Proceedings of the Second

Clinical ACTH

Conference

Volume I—Research

The *second* conference on **ACTH**
was sponsored by ARMOUR & COMPANY Chicago
and held at the Palmer House Chicago
on *December 8 and 9 1950*

Proceedings of the Second Clinical ACTH Conference

Volume I—Research

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THE BLAKISTON COMPANY
New York Philadelphia Toronto
1951

PRINTED IN THE UNITED STATES OF AMERICA
BY KINGSPORT PRESS INC KINGSPORT TENN

Introduction

During 1950 the availability of ACTH increased markedly to the end that it was freely obtainable for both investigative and therapeutic use which resulted in continued investigation on a greatly increased scale In consequence The Armour Laboratories concluded that it would be constructive to call a Second Clinical ACTH Conference This was held in Chicago December 8 and 9 1950 and a total of 102 papers were presented

This amount of material presented at the conference turned out to be too large to publish in a single volume and in consequence the Proceedings are published in two parts—a first volume designated as Research consists of papers dealing primarily with research at the more fundamental level a second volume designated as Therapeutics contains papers dealing primarily with the clinical aspects of the problem In view of the fact that this whole new era of medicine is still at the investigative stage all of the papers are really research presentations However most of the papers presented at the conference contain both clinical and fundamental observations relating to adrenal cortical stimulation under different physiologic or metabolic situations and in different disease states In this light the division is arbitrary but unavoidable and papers on the same disease may appear in both volumes depending upon the major emphasis in the subject matter presented

The reports contained in the Research volume deal primarily with the more fundamental aspects of ACTH and adrenal cortical function as well as the effects of adrenal corticoids on different tissue systems under varied physiologic and metabolic conditions either in the normal human being or in different disease states In most instances the clinical observations are coincidental to biochemical physiologic or metabolic studies or to investigations attempting to elucidate mechanisms that may be in operation in different physiologic or disease states

The papers presented in the Therapeutic volume on the other hand relate primarily to the effects of ACTH in the treatment of different disease syndromes although most of the reports contain observations of a fundamental character An attempt is also made in this second volume to summarize the results to date in the treatment of different disease syndromes with ACTH in order to obtain a clearer picture concerning the therapeutic use of ACTH

Each volume of the Proceedings of the Conference contains in *addition to its own Table of Contents* a *Table of Contents of the* other volume An examination of the Tables of Contents of the two volumes reveals a substantial extension of knowledge concerning the role of the adrenal gland in health and disease and the practical use of ACTH in treating different disease syndromes In this connection the contributors and many other investigators working in this field are to be congratulated for the scope and quality of work that has been undertaken during 1950

On the other hand it is still fair to say that the scope of the work remaining to be done in resolving rationally the problems raised by this new era of medicine is far from clear although admittedly of *major magnitude However the challenge is great and the broad* implications on health and disease in general are profound It is therefore reasonable to forecast that the imagination the manpower and the facilities will be forthcoming to solve the problems raised to the credit of the profession and the benefit of the public at large

In the interval it is reassuring that many otherwise ill or incapacitated patients can be rehabilitated into competent and functional units of society by the proper and judicious use of ACTH

In the volume Proceedings of the First Clinical ACTH Conference well deserved commendation was given *to the many* investigators for the truly vast amount of excellent research work that had been accomplished in the short time that the limited quantities of ACTH were available This was particularly true of studies relating to the physiologic and metabolic function of the adrenal gland in the normal human being and in different disease states

It was reported in that volume that increased adrenal cortical function altered the clinical course of a number of disease syndromes but the point was made *at that time that intensive investigation* over a wide area of medicine would be required to develop a reasonable pattern of the role and function of the adrenal gland in health and illness in general It was likewise forecast that a tremendous amount of work would be required to arrive at valid conclusions concerning the effect of adrenal cortical stimulation in various disease syndromes and even more important to elucidate the physiologic and metabolic abnormalities which may be concerned in the many apparently widely divergent disease states which are altered by adrenal cortical stimulation

In drawing up the program of the Second Clinical Conference the major emphasis was on the investigation of new areas of fundamental and clinical sciences or on new approaches *to well-defined* problems On the other hand an attempt was also made to arrive at an overall evaluation of the therapeutic effectiveness of ACTH in

diseases previously reported This latter was done by holding symposia on different related disease syndromes

An attempt was made to keep the conference informal in order to allow a free exchange of ideas as was the case in the first conference in consequence of which considerable discussion arose

Many of the studies have further clarified the function of the adrenal gland in physiology and metabolism Many other studies in progress have continued to explore the role of the adrenal cortex in disease in general and still others have more clearly defined the use of ACTH in the treatment of specific disease syndromes

The authors and discussers in the second conference had an opportunity to revise their presentation and discussion remarks In order to expedite publication the manuscripts and discussions are published without further significant editing or correction by the authors the editor or the publisher

In view of the preliminary and tentative nature of many of the results reported in this volume the contents herein may not be quoted without the prior consent of the author and the publisher

ACKNOWLEDGMENT

The editor has been requested by the members of the conference to extend their thanks and appreciation to Mr F W Specht President of Armour and Company and his colleagues for their foresight and generosity in making the Second Clinical ACTH Conference possible

The editor should also like to express his personal appreciation to Mr George Josh for arranging the many details which resulted in a smooth running conference Finally Miss Marjorie Cullins deserves particular commendation for the excellent manner in which she organized the many details relating to the program the manuscript and the discussion remarks Her work has in a large measure made early publication possible

The Blakiston Company personnel and its manufacturing associates under the direction of Mrs Eunice Stevens Associate Medical Editor deserve special thanks for the rapidity with which these volumes have been made available to the reader

JOHN R MOTE

Chicago
March 1951

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PART I

GENERAL ADRENAL CORTICAL PHYSIOLOGY AND ADRENAL CORTICAL STEROID SECRETION AND EXCRETION

1

Studies with Labelled ACTH Preparations* †

Martin Sonenberg ‡ Albert S Keston and William L Money ‡

MEMORIAL CENTER FOR CANCER AND ALLIED DISEASES CORNELL UNIVERSITY MEDICAL COLLEGE AND NEW YORK UNIVERSITY-BELLEVUE MEDICAL CENTER NEW YORK

We have previously reported[1,2] the feasibility of labelling pituitary proteins with radioactive iodine (I^{131}) The specificity of an I^{131} labelled ACTH preparation for the adrenal cortex of the rat was demonstrated by radioautography (Figure 1) as well as by direct radioactivity measurements of the gland The rapid disappearance of radioactivity from the adrenal was demonstrated The presence of radioactivity in the thyroid was assumed to be due to iodine which was broken off the labelled ACTH

The technique of trace labelling[2] was used to prepare the radioactive hormone preparations employed in this study In this method the average ratio of radioactive substituent molecules per protein molecule is a small fraction When small traces of the labelling reagent are used there is greater likelihood that biological activity will be retained for it becomes very improbable that the same molecule

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* This work was carried out during a tenure of a fellowship in Cancer Research of the American Cancer Society and supported in part by a grant in aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council This study was supported in part by the Office of Naval Research and the Atomic Energy Commission under Contract no N8 onr 669

† We are indebted to Dr Rulon W Rawson for his cooperation and interest in this investigation We are also grateful to Dr Wendell C Peacock and Dr Patrick Fitzgerald for their assistance

Miss Jean Fager Miss Virginia Lucas Miss Eva Simmel and Mr Jerome Weinstein contributed valuable technical assistance in these studies

‡ Fellow in Cancer Research of the American Cancer Society

WOE 18AR was considered to have 80 times the activity of the LAIA standard while the J13801 preparation contained 2 2 times the activity of the LAIA standard) This corresponded to a ratio of active material of approximately 10 1 One minute after an intracardiac injection these animals were sacrificed by exsanguination and then perfused with 100 cc of isotonic saline The adrenals were then removed and assayed for radioactivity

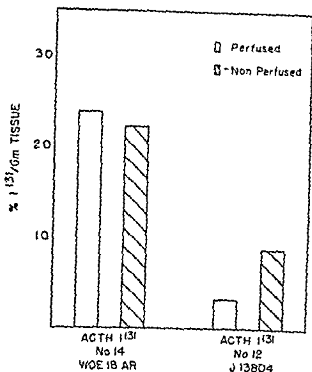


FIG 2 Relationship of concentration of radioactivity in the adrenals after the administration of I[131] labelled ACTH preparations of varying biological potency

Comparisons are made (Figure 2 and Table I) between the concentrations of radioactivity expressed as per cent of the administered dose which localizes in a gram of tissue There was an absolute increase with a ratio of approximately 8 1 in the degree of localization of radioactivity of the more biologically potent preparation (Armour WOE 18AR) over a similarly labelled preparation (Armour J13804) of modern hormonal activity This is in good agreement with the estimated dosage ratio of active substance in these preparations of 10 1

Seven animals were treated in a similar manner with the two



FIG 1 Radioautograph of an adrenal gland from an animal that had received an I^{131} labelled ACTH preparation

which has been labelled will have been oxidized or reacted in any other way The radioactive hormone preparations used in these experiments were made by iodination with small amounts of I^{131} relative to the amount of protein

CORRELATION OF BIOLOGICAL ACTIVITY WITH LOCALIZATION OF RADIOACTIVITY AFTER THE ADMINISTRATION OF A LABELLED ACTH PREPARATION

An attempt has been made to obtain further evidence of the specificity of labelled ACTH preparations for the adrenal glands For this purpose comparisons were made between biological potency and localization of radioactivity

Six normal adult male Sprague Dawley rats were injected intracardially with labelled ACTH* preparations of different biological potencies Each rat received either 150γ of the Armour WOE 18AR preparation or 500γ of the Armour J13804 preparation (Preparation

* All the ACTH used in this study was kindly supplied by the Armour Laboratories

WOE 18AR was considered to have 80 times the activity of the LAIA standard while the J13801 preparation contained 2 2 times the activity of the LAIA standard) This corresponded to a ratio of active material of approximately 10 1 One minute after an intracardiac injection these animals were sacrificed by exsanguination and then perfused with 100 cc of isotonic saline The adrenals were then removed and assayed for radioactivity

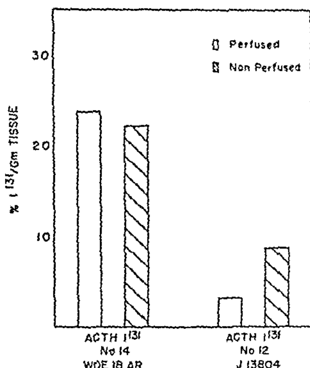


Fig 2 Relationship of concentration of radioactivity in the adrenals after the administration of I^{131} labelled ACTH preparations of varying biological potency

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Table I

Preparation	*Rat wt gms*	*Adrenal wt mgms*	*Adrenal* I^{131} *cpm*	*%* I^{131} *Adrenals*	*%* I^{131}/gm *Adrenals*	*Ave %* I^{131}/gm *Adrenals*
			WOE 18AR			
Perfused	213	29 2	3761	0 071	2 43	2 37
	273	31 0	3817	0 072	2 32	
Non Perfused	222	33 0	5591	0 106	3 21	
	248	33 5	3312	0 063	1 88	2 22
	254	48 3	4066	0 076	1 58	
			J 13804			
Perfused	244	38 1	271	0 013	0 34	
	238	36 0	235	0 011	0 31	0 31
	260	40 7	240	0 011	0 27	
	212	36 2	250	0 012	0 33	
Non Perfused	240	48 3	655	0 031	0 64	
	217	29 0	653	0 031	1 07	0 87
	255	37 2	816	0 039	1 05	
	224	35 5	545	0 026	0 73	

CPM is counts per minute
Last 3 columns show % of I^{131} in ACTH sample used which localized in the adrenal glands

labelled ACTH preparations with the exception that the animals were not perfused following exsanguination

When comparisons are made (Figure 2 and Table I) between the degree of localization of radioactivity in the adrenals there is but a factor of approximately 2 5 1 in favor of the more biologically potent ACTH preparation This is in poor agreement with the ratio of approximately 10 1 anticipated from the hormonal activities On perfusion the more active preparation seemed to lose no appreciable radioactivity whereas the less active preparation lost over 60% of the original radioactivity This suggests that much of the material lost by perfusion was non specific for the adrenal

It is to be expected that when hormone preparations of low biological activity are used the iodinated non hormonal proteins would create background which would tend to mask any specific binding Hence in future experiments we shall use if possible the most biologically active preparations available

Attention will be focussed mainly on that fraction of the radioactivity in a tissue which resists perfusion or which survives the fixation procedures used in preparation for radioautographs

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DISCUSSION

DR PAUL STARR (University of Southern California School of Medicine Los Angeles) We have been working on radioactive TSH (pituitary thyroid stimulating hormone) at the University of Southern California Is there any possibility that not all of the molecules of the ACTH referred to by Dr Sonenberg in his work are actually iodinated? In other words only 10 per cent of them could be iodinated with the isotope and go along to the gland but the stimulation of the gland be due to the uniodinated ACTH

In relation to the fact stated i e that there was a radioautograph of the thyroid—in other words that some of the iodide went there my question is as follows Is that due to unbound isotope or is it conceivable that some of the labelled ACTH also went to the thyroid? If so did the thyroid show evidence of inhibition as one would expect? Not as one would expect but as one would hope?

Next is it possible—(I am really asking for the work on TSH [pituitary thyroid stimulating hormone])—that any iodinated TSH in turn has gone to the adrenal cortex?

DR MARTIN SONENBERG In these studies Dr Starr we are only measuring radioactivity The possibility that some of the molecules may not be iodinated and are stimulating the gland is very possible The only way we can approach the problem is to run controls and notice that other substances do not behave as I^{131} labelled ACTH does

As far as the radioactivity in the thyroid is concerned I may have been a little brief on that point We assume it is due to inorganic iodide inasmuch as when inorganic iodide is given thirty seconds after it is given intravenously you can pick up significant amounts of radioactivity in the thyroid However after the administration of labelled ACTH there is a lag of about eight minutes presumably the period when the ACTH is being broken down Then the curve in the thyroid rises very precipitously We have concluded from this that it is the inorganic iodide which is localizing not the radioactive protein Furthermore the ACTH preparations used had virtually

no assayable TSH (pituitary thyroid stimulating hormone) in them

It is possible that TSH either labelled or non labelled may localize in the adrenals Our studies with labelled TSH have not helped elucidate this point Incidentally we feel that TSH is quite inhomogeneous of all the pituitary fractions available to us

2

Metabolic Effects of Small Molecular ACTH A Preliminary Report*

Peter H Forsham A Renold J B Lesh

PETER BENT BRIGHAM HOSPITAL AND HARVARD MEDICAL SCHOOL BOSTON AND THE ARMOUR LABORATORIES CHICAGO

MATERIAL

To date small molecular ACTH has been given three clinical trials[1 2 3] In all the material was prepared by Li s peptic digestion method The present communication deals with the use of a more highly purified preparation isolated by Lesh and his collaborators of the Armour Laboratories[4] The material represents the high potency fraction 12 obtained from standard material fractionated by pH ad justment peptic hydrolysis and counter-current distribution of the trichloracetic acid soluble fraction following pepsin treatment This preparation was made up in propylene glycol solution 1 ml of which contained 0 4 mg of solid of which 0 34 mg was organic material Assay by the method of Sayers Sayers and Woodbury[5] as modified by Munson revealed a potency of 35 mg in terms of standard LA 1 A per ml of the propylene glycol solution In terms of the known or ganic content this ACTH preparation was thus approximately one hundred times the standard potency

ASSAY IN MAN

The method of Hills and Thorn[6] utilizing a fifty percent fall in circulating eosinophils as an end point was employed with the results shown in Table I There was a very wide scatter of the results How ever the potency of the material seemed to be certainly as high and most probably higher than that determined by the rat assay

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The authors wish to extend their thanks to Dr George W Thorn for his con tinued interest and advice in this study They also wish to extend their appreciation to Dr Thomas F Frawley for setting up and supervising the formaldehydogenic steroid determinations and to Miss Ann L Grimes Mrs Lois Nellis Miss Claire Preston and Mr William J Reddy for their technical help

Table I

EOSINOPHIL ASSAY OF POLYPEPTIDE ACTH

Dose by Animal Assay	*Normal Subjects*	*Eosinophil Fall %*	*Dose by Human Assay*	*Factor*
2 5 mg	D W	53	5 0 mg	2 0
2 5 mg	C L	33	2 6 mg	1 0
2 5 mg	D L	18	1 8 mg	0 72
1 25 mg	D S	63	10 1 mg	8 0
1 25 mg	D H	35	2 7 mg	2 2
1 25 mg	D S	26	2 2 mg	1 8
			MEAN	2 6

METABOLIC STUDIES

INTRAMUSCULAR ADMINISTRATION

C B a twenty year old normal English student served as a subject He was placed on a constant diet and constant fluid intake consisting of C 280 P 135 F 162 Na 140 mEq K 120 mEq fluid 2000 ml per day Throughout the period the bowel habits were regular Urine was collected daily and bloods were obtained for studies at appropriate time intervals Methods employed were those previously used by this group in other studies[7] Sodium and potassium determinations were done by internal standard flame photometry

Experimental Set Up

The polypeptide ACTH preparation ACTH No R 256023 *(henceforth called PPA) was administered intramuscularly every six* hours after diluting the propylene glycol solution with an equal volume of saline within twelve hours before its use leaving the syringe in the icebox throughout The ACTH No J 8006 (henceforth called RA) was administered similarly but merely dissolved in saline so as to have a final concentration of 20 mg per cc The dosage administered being made comparable on the basis of assuming PPA to be 100 times standard potency *are clearly indicated in the diagrams in terms of* the Armour Standard LA I A The plan set up was to first give the PPA in increasing dosage to then have an interim period with no treatment and subsequently a period of RA with doses comparable to those of the PPA previously given

Results

A *Miscellaneous Changes*

The fall in circulating eosinophils was qualitatively comparable with the two ACTH preparations used (Fig 1) but quantitatively

COMPARISON OF POLYPEPTIDE AND REGULAR ACTH

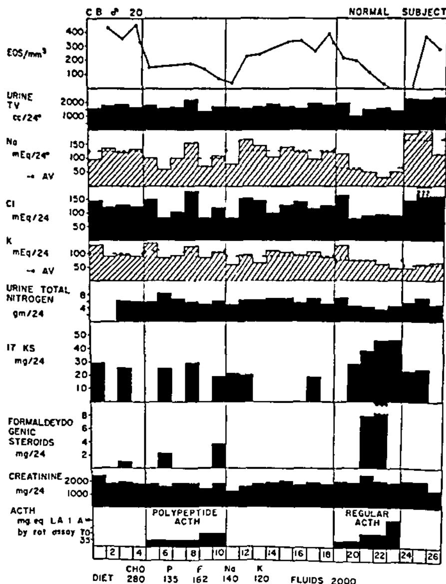


Fig 1

more marked with RA Serum uric acid fell 1 5 mg on the PPA and 2 mg % on the RA while there was a slight rise in urinary output only on RA

Both preparations produced changes in carbohydrate metabolism (Fig 2) with a minor rise in fasting blood sugar in both instances

COMPARISON OF POLYPEPTIDE AND REGULAR ACTH

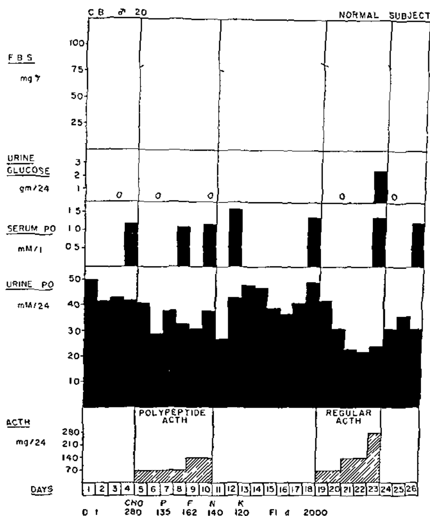


Fig 2

more marked on RA Urinary phosphorus fell more on RA presumably as a consequence of increased liver glycogen deposition

IV glucose tolerance tests using 0 5 grams per kilogram intravenously in 20% solution given over a thirty minute period on the last day of 140 mg of ACTH showed a moderately diabetic type on PPA and more so on RA in both instances accompanied by a less than the usual 20% fall in *serum inorganic phosphorus (Fig 3) This would* indicate peripheral under utilization of the glucose administered

B *Electrolyte Changes*

No significant water retention was obtained on PPA whereas there was water retention with rebound on RA (Fig 1) There was a sig

nificant sodium retention on PPA with a rebound to the previous level On RA the sodium retention was more marked with a rebound beyond the control level Changes in urinary chloride followed those of sodium Potassium changes were negligible on PPA and there was a slight potassium retention on RA There were no significant changes in serum electrolytes on either preparation

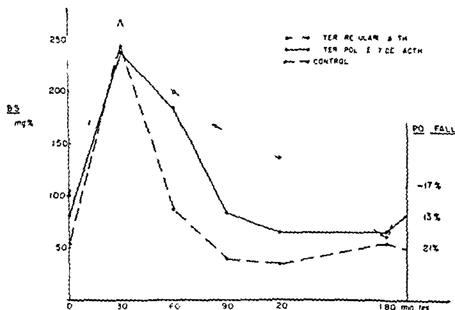


Fig 3

Summary

In general PPA showed the same qualitative effect as RA in these experiments When comparing the changes produced quantitatively PPA appeared approximately twenty five times as potent as RA An exceptional reaction was the fall in 17 ketosteroids on PPA which suggested a number of possible explanations

(1) That a minimal stimulation of the adrenal cortex as was obtained by the dosage of PPA used might fail to produce a rise in 17 ketosteroids

(2) That PPA might stimulate only the Compound F like steroids of the adrenal cortex which would then suppress endogenous ACTH sufficiently to reduce the normal 17 ketosteroid excretion

The second point was ruled out by an intravenous experiment based upon the use of 0 4 mg of PPA

INTRAVENOUS ADMINISTRATION

Gordon[8] had shown previously that intravenously administered ACTH given continuously over a prolonged period exerts a much higher biological effect than repeated intramuscular injection in man Ingle[9] had established that ACTH given intravenously as a continuous drip to rats led to the formation of truly gigantic adrenal cortices With these facts in mind a simple experiment was set up consisting of the administration of ACTH in saline continuously over an eight hour period

T V a twenty year old male student served as a subject He was placed on a constant diet and constant fluids consisting of C400 P 150 F 240 Na 200 mEq Γ 115 mEq and fluids 2000 ml per day

Experimental Set Up

After five days on constant diet the patient was given 10 mg of RA in 480 cc of 0 72% NaCl in water intravenously over a period of eight hours The solution for infusion was made up in hourly portions of 60 cc from a concentrate in order to avoid prolonged exposure at room temperature During the infusion there was no change in meals which were administered in the usual fashion having had the contents of the intravenous infusion deducted during the twelve hour period comprising the infusion Blood samples were drawn at appropriate intervals Twelve hour urine collections were obtained throughout from 7 A M to 7 P M and 7 P M to 7 A M The infusion was started at 8 A M After a four day interval the identical procedure was repeated injecting 0 5 mg of PPA

Results

A *Miscellaneous Changes*

The eosinophil fall while comparable with both ACTH preparations occurred somewhat earlier and was more marked on PPA The total white count rose with both to the same extent and returned to normal within twenty four hours There was a marked intermittent glycosuria with both preparations (Fig 4)

B *Electrolyte Changes*

Urine excretion increased with both preparations of ACTH in excess of the volume of fluid given intravenously Urinary sodium excretion showed no change during the twelve hours comprising the infusion on RA but a definite decrease on PPA During the ensuing twelve hours there was a marked fall in urinary sodium excretion

IV INFUSION OF POLYPEPTIDE AND REGULAR ACTH

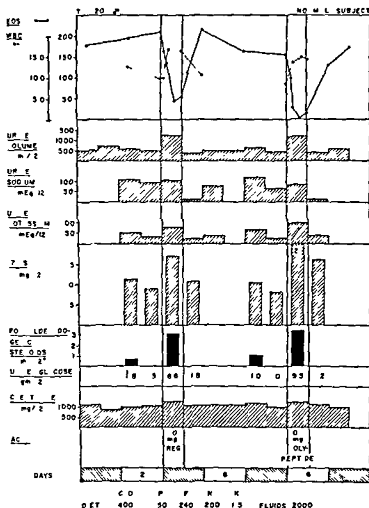


Fig 4

associated with a fall in the urinary concentration of this ion in both instances The excretion of chloride followed that of sodium Urinary potassium excretion showed a definite rise during the first twelve hours on both preparations more marked on PPA There was no rebound retention (Fig 4)

C *Urinary Steriod Excretion*

The formaldehydogenic steroids showed a five fold increase on both preparations during the twelve hour period including the in

fusion The excretion of 17 ketosteroids went from 11 5 to 17 2 mg per twelve hours on RA and from 10 6 to 21 1 mg per twelve hours on PPA (Fig 4)

Summary

Essentially identical effects throughout were observed with 10 mg of regular ACTH and 0 4 mg of polypeptide ACTH when either were given intravenously over an eight hour period From practically all the changes the ratio of potency worked out to be the same mak ing the purified peptide preparation 25 times more potent than Standard LA 1 A

The intravenous effectiveness of RA appeared stepped up five fold that of PPA eight fold when compared to the intramuscular route suggesting greater in situ inactivation of the purer form of ACTH and/or a more rapid absorption into the blood stream with *less efficient adrenal cortical stimulation*

GENERAL DISCUSSION

PPA appeared to lead to all the changes attributed to date to the ACTH preparations of larger molecular size with the exception of a rise in 17 ketosteroids when the material was given intramuscularly every six hours However by the intravenous route PPA led to a marked rise in 17 ketosteroids

The effect of PPA given intravenously for eight hours is remark able if one considers that only 50 gamma of biologically active ma terial represent the theoretical maximum stimulating the adrenal cortices during the period of one hour or somewhat less than 1 gamma per minute These changes might be thus classified as truly hormonal in character

The potency of PPA judged by overall metabolic changes was only one quarter of that obtained by animal assay or the eosinophil in man *Both are based on the effects of a single short activation of* the adrenal cortex by the ACTH administered Metabolic changes on the other hand rely for their magnitude and duration on con tinued or repeated adrenal stimulation which may well differ for ACTH preparations of various molecular sizes though identical in short term action on the adrenal cortex

The data presented clearly demonstrate that the polypeptide ACTH is capable of producing the cardinal effects of adrenal cor tical stimulation as previously shown in man for the large molecular preparation [7] viz Eosinopenia and changes in carbohydrate metab olism sodium and chloride retention with potassium loss and a rise

in 17 ketosteroid excretion in quantities of less than 1 gamma per minute intravenously

SUMMARY

A preparation of polypeptide ACTH which assayed about one hundred times the standard preparation LA 1 A by the rat ascorbic acid assay and the human eosinophil assay was shown to be twenty five times as potent as the standard LA 1 A preparation in overall metabolic activity

The polypeptide ACTH preparation produced all the hematological and metabolic changes previously demonstrated with the use of the commercially available preparations of Armour ACTH

Marked changes were produced with as little as 1 gamma per minute when administered intravenously over an eight hour period

The small molecules appear to be biologically equivalent to their larger progenitor

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DISCUSSION

DR CHARLES RAGAN (Presbyterian Hospital and Columbia University College of Physicians and Surgeons New York) We have some material that has been prepared in approximately the same way as that done by Dr Lesh This was made for us by Dr Folkers at Merck We have used this on several patients with rheumatoid arthritis

One characteristic feature of this preparation has been that during the process of hydrolysis most of the antidiuretic principle has been removed and in our studies we have been impressed that with adequate control of the symptoms of arthritis there has been very

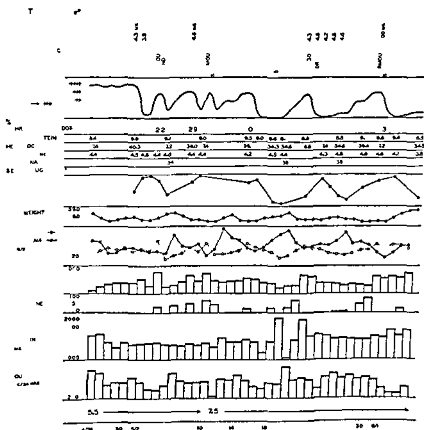


FIG 5

little sodium retention with these small molecular preparations In Fig 5 is shown such a case He received a rather large amount of degradation product of ACTH 50R1777 in a dose of approximately 40 mgm a day There was a minor degree of sodium retention

A smaller molecule 50R3033 in a dose of 4½ mgm in a 24 hour period was followed by a remission of symptoms of arthritis but with relatively no change in sodium excretion With a similar remission brought about by Armour standard ACTH there was a significant weight gain and moderate sodium retention

DR SHELDON MARGEN In the original report of the metabolic effects of peptides derived from ACTH which we presented at this meeting one year ago (refer Proceedings of First ACTH Conference for data) we noted a greater sodium retaining effect of the peptide than of the whole ACTH in relation to the amount of water retained and assumed that the discrepancy could be attributed to posterior pituitary contamination of the whole ACTH The increase in 17 ketosteroid excretion was very marked with both materials

DR J S L BROWNE I would like to point out that there is a naturally occurring circumstance that of pregnancy in which there is a marked discrepancy between formaldehydogenic urinary corticoids or biologically active corticoids and the 17 ketosteroids true 17 ketosteroids not rising and the other materials rising quite markedly as shown by Dr Venning

Other circumstances in the body beside the nature of the ACTH administered may affect this type of response

DR WILLIAM Q WOLFSON (University Hospital Ann Arbor) Our studies on long acting ACTH preparations have forced us to do bio assay studies comparing the metabolic effects of different ACTH preparations In the process we have fallen into most of the available traps some of which are available to confuse almost any study of this type

Table II indicates that some metabolic indices change so rapidly in response to a constant daily dosage of ACTH that they simply are of no value in comparative bioassay This figure summarizes average data from 26 subjects given 40 mg doses of Adactar on each of two successive days during which 24 hour urine collections are made Results are compared with values in control 24 hour urines In general the test is similar to the 48 hour aqueous ACTH test introduced by Forsham and Thorn

On the first test day the metabolic index which shows the largest change is the urine potassium/urine sodium ratio This however fades out very rapidly and is far less elevated on the second day With

Table II

EFFECT OF TWO SUCCESSIVE 40 MG DOSES OF ADACTAR IN 26 SUBJECTS

	% of Control Value	
	First Test Day	*Second Test Day*
URINE URATE/URINE CREATININE	113%	110%
URATE CLEARANCE/CREATININE CLEARANCE	129%	128%
URINE POTASSIUM/URINE CREATININE	115%	100%
URINE POTASSIUM/URINE SODIUM	223%*	106%*
URINE HISTIDINE/URINE CREATININE	135%	150%
URINE 17 KETOSTEROID/URINE CREATININE	151%	163%

* Median values

doses of this size potassium diuresis is much less prominent even in the first 24 hour urine than in a four hour test and is almost absent on the second day Clearly counter regulatory factors so largely in fluence the changes in these electrolytes that they are of little value in quantitative assay work

Two metabolic functions show a progressive increase during the 48 hour procedure These are the urine 17 ketosteroid/creatinine ratio and histidine/creatinine ratio The values of urine urate/cre atinine and urate clearance/creatinine clearance occupy an inter mediate position between the type of change shown by the electrolytes and the type of response shown by ketosteroids and histidine

These patterns of change appear to be fundamental to the indices studied and have been observed to occur in gout patients in rheu matoid arthritis patients and in controls Any qualitative differences between various groups of subjects which we have found are poorly established and may disappear with further experience

A second point deals with the problem of evaluating the results of studies in which one ACTH preparation is given for a particular period and a second preparation is given after a brief rest period In one such study in our series aqueous ACTH was given in the first period and Adactar in the second period The results were almost identical with those Dr Forsham has reported here no ketosteroid increase in the first period and a marked increase in ketosteroid ex cretion in the second period Later studies with the same lot of Adac tar showed that when compared with aqueous ACTH by other pro cedures it gave almost identical increases in urine 17 ketosteroid

As a result we have come to question whether successive com parison with an interspersed rest period is actually a valid compara tive assay procedure The administration of ACTH followed by its withdrawal produces profound changes in body physiology and we

do not yet possess any reliable way of determining the time at which the subject has returned to a basal status

DR JOSEPH E WARREN (House of Good Samaritan and Harvard Medical School Boston) Dr Forsham s report on the potency of these small molecules is fascinating but I am worried a bit about the dependability of these sequential assays

We have been doing some studies of the clinical and metabolic effects of short (4 day) courses of ACTH in low grade rheumatic fever and have noted that there is often a variable polyphasic recovery period of five to fifteen days duration At first after omission of ACTH there may be a rebound of the eosinophiles and of other indices of S hormone activity This rebound reaches its peak in anywhere from two to ten days and may be followed by a second phase of endogenous ACTH production which in turn may be slightly excessive before stabilization occurs at the previous or a normal baseline

Since the peak of rebound may be delayed to five or six days it is not unlikely that a second course of exogenous ACTH at an eight day interval may concide with a period of increasing endogenous production of ACTH and of increased sensitivity of the target organ

DR PETER H FORSHAM The suggestion that endogenous ACTH production might change serial experiments is well taken However in our first experiment we had a one week interval which should have allowed the return of basal endogenous ACTH production In the intravenous experiment small amounts of the large molecule were given four days after the small molecular preparation and it is possible that the pituitary was in active rebound at the time of the second experiment

The existence of different maxima for various indices of adrenal cortical activation for different preparations of ACTH is very likely It is of interest that when ACTH was given intramuscularly this was found to be true but effects following intravenous ACTH could be practically superimposed for the large and small molecule

There was no antidiuretic effect of the small molecular fractions but actually a diuretic effect was obtained with intravenous administration, with marked sodium retention

The results reported with the small and rather homogenous ACTH are identical with those reported by Dr Li and his colleagues on two previous occasions Barring contamination with an ultra potent large molecule it appears that this preparation with an average molecular weight ranging from 1000 to 1500 shows all of the effects of large molecular ACTH

3

Concerning the Probability that There Are at Least Two Adrenocorticotrophic Hormones in the Human Being*

N B Talbot, M S Wood, A M Campbell, E Christo, and A S Zygmuntowicz

MASSACHUSETTS GENERAL HOSPITAL AND HARVARD MEDICAL SCHOOL BOSTON

At last year s conference data was presented which indicated that the urinary 11 17 oxycorticosteroid (11 17 OCS) output per square meter of body surface (m^2) per day of normal infants children and adults was essentially the same At the same time it was shown that the urinary 17 ketosteroid (17 KS) output per m^2 per day was low during the first approximately 8 years after which it rose steadily until adult levels were attained at about 18 years The present paper describes studies undertaken in an effort to gather information con cerning the possible nature of the factor or factors responsible for this qualitative change in adrenal cortical activity during adolescence

The investigations reported here are divided into two parts The first were designed to determine whether the responsiveness of the ad renal cortex to a standard ACTH stimulus varied with age The second had the purpose of determining whether the response of the human adrenal cortex to endogenous ACTH secreted in response to stress might differ appreciably from that observed following ex ogenous ACTH administration

METHODS AND MATERIALS

The urinary steroid analyses were performed with the aid of methods reported elsewhere[2,3] The 17 ketosteroid procedure in cludes steps designed largely to eliminate errors or overestimation due to common interfering chromogens Likewise the 11 17 oxycor ticosteroid procedure includes an extensive purification procedure which appears to reduce errors of overestimation due to non steroidal

* This work was supported by grants from the Commonwealth Fund of New York from the American Cancer Society and by generous allotments of ACTH from Armour and Company

reducing agents to a minimum Because of these factors 17 ketosteroid and 11 17-oxycorticosteroid values obtained with the aid of these analytic methods tend to yield lower values than are given by a number of other methods in common use

RESULTS AND COMMENTS

1 Effect of exogenous ACTH upon urinary steroid excretion

Figures 1 2 and 3 describe the changes in urinary 11 17 oxycorticosteroid and 17 ketosteroid excretion noted when ACTH was given to 3 infants 3 children and 3 adults In the infants (Figure 1) ACTH induced a greater absolute increase in 11 17-oxycorticosteroid excretion than it did in 17 ketosteroid excretion By contrast in the children (Figure 2) and adults (Figure 3) ACTH treatment caused a greater absolute increase in 17 ketosteroid than in 11 17 oxycorticosteroid values

The magnitude of differences in response are indicated in Table

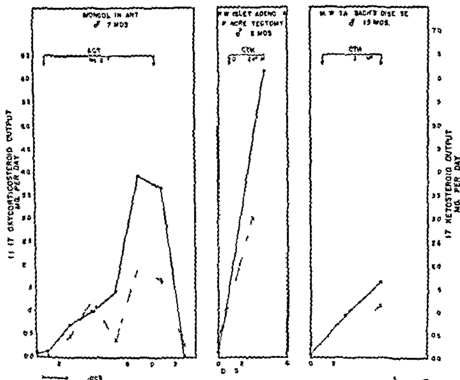


Fig 1 *Effect of Armour ACTH administration upon the urinary 11 17 oxycorticosteroid and 17 ketosteroid excretion by 3 infants* Results expressed as absolute milligrams *per* 24 hours ACTH dosages given as mg per square meter of body surface (m^2) per day

I Note in the middle columns that infants attained considerably higher absolute 11 17-oxycorticosteroid values than either children or adults Contrariwise adults attained very much higher absolute 17 ketosteroid values than infants and children Nonetheless the younger subjects excreted appreciable quantities of 17 ketosteroids while under the influence of ACTH The data of the right hand

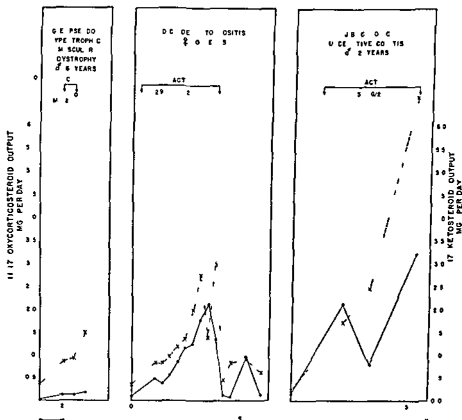


FIG 2 *Effect of Armour ACTH administration upon the urinary 11 17 oxycorticosteroids and 17 ketosteroids of 3 children* The design of this figure is the same as that of Figure 1

columns reveal that ACTH caused a much greater per cent increase in 11 17 oxycorticosteroid than in 17 ketosteroid excretion above control values in all three groups of individuals As might be expected from these findings Figure 4 shows that ACTH treatment rarely caused the urinary 17 ketosteroid output to rise even to adult normal levels without inducing marked hyper 11 17-oxycortico steroiduria This was universally observed in persons under 15 years of age and in over half of those over 15 years old

Comments Administration of Armour ACTH apparently causes a

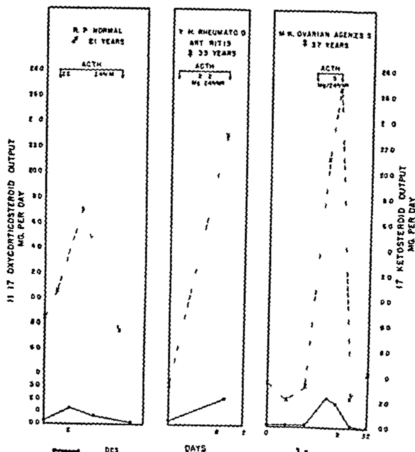


FIG 3 *Effect of Armour ACTH administration upon the urinary 11 17 oxycorticosteroid output of 4 adults* The design of this figure is the same as that of Figures 1 and 2

very large increase in urinary 11 17-oxycorticosteroid excretion in individuals of all ages and especially in infants The latter fact is remarkable in view of the small size of the infant (about 0 3 to 0 5 m^2) compared to the adult (about 1 7 m^2)

Available information indicates that the capacity of the adrenal cortices to secrete 11 17-oxycorticosteroids wanes rapidly in the absence of ACTH[4] Hence it seems reasonable to infer from the foregoing data that the anterior pituitaries of the infants children and adults alike must normally produce appreciable quantities of an ACTH capable of stimulating 11 17-oxycorticosteroid secretion

Administration of ACTH also causes a marked and approximately equivalent per cent increase in urinary 17 ketosteroid excretion in individuals of all ages irrespective of the intensity and duration of

1 Note in the middle columns that infants attained considerably higher absolute 11 17 oxycorticosteroid values than either children or adults Contrariwise adults attained very much higher absolute 17 ketosteroid values than infants and children Nonetheless the younger subjects excreted appreciable quantities of 17 ketosteroids while under the influence of ACTH The data of the right hand

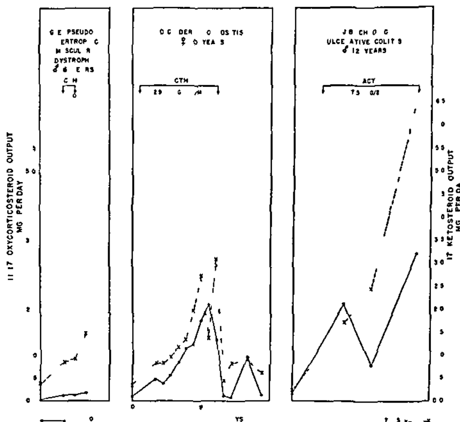


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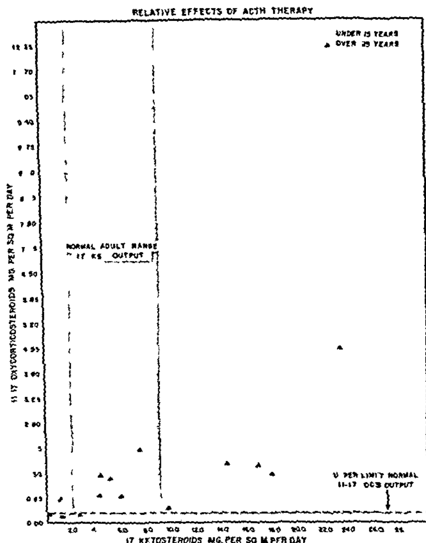


FIG 4 *Relations between urinary 11 17 oxycorticosteroid and 17 keto steroid excretion values of miscellaneous endocrinologically normal per sons to normal values during periods of ACTH treatment* Note that in this figure steroid excretion values are expressed as mg per m² per day The horizontal shaded area near the bottom of the figure gives the normal range for 11 17-oxycorticosteroid output by healthy persons The vertical shaded area gives the normal adult male plus female range for 17 keto-steroid excretion

dosage This suggests that the 17 ketosteroid excretion values of in fants and children normally are very nearly zero because their adrenal cortices are not being stimulated by an ACTH capable of inducing normal adult type 17 ketosteroid precursor production

Table I

AVERAGE EFFECT OF ACTH UPON URINARY 11 17 OXYCORTICOSTEROID AND 17 KETOSTEROID OUTPUT OF INFANTS CHILDREN AND ADULTS

Type of Subject	Average Control		Average Maximum Increase		Average Percent Increase		A/B
	11 17 oxycorticosteroids mg /day	17 ketosteroids mg /day	11 17 oxycorticosteroids mg /day	17 ketosteroids mg /day	11 17 oxycorticosteroids (A)	17 ketosteroids (B)	
Infants	09	24	3 9	1 8	4340	750	5 8
Children	08	32	1 5	2 9	1870	905	2 1
Adults	32	4 1	2 8	17 9	875	435	2 0

ketosteroid production to be relatively undeveloped during infancy and early childhood and to enlarge during adolescence Blackman has described histologic changes in the zona reticularis which largely fulfill these theoretical requirements [8]

2 Effect of Stress upon Urinary Steroid Excretion

Figures 5 and 6 present observations on two patients who did not receive exogenous ACTH but who underwent stress sufficient to cause alterations in endogenous ACTH production as measured by changes in urinary steroid excretion The first was an adult who suffered severe thermal burns the second was a 19 year old diabetic Mongol who had a series of insulin reactions Observe that in both

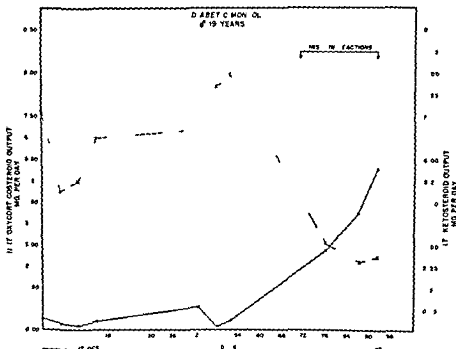


Fig 6 *Urinary 11 17 oxycorticosteroid and 17 ketosteroid excretion by a diabetic Mongol before (days 0 to 72) and during (days 72 to 93) a period of stress occasioned by insulin reactions* Results expressed as mg per 24 hours From "The effect of various regimes of insulin therapy upon adrenal cortical function in the diabetic patient" (In preparation) by McArthur J W Chao H C MacLachlan E. A Morrill M F Zygmuntowicz A S Wood M S Campbell A M., Talbot N B and Butler A M

These findings make it appear likely that the human anterior pituitary secretes one type of ACTH which stimulates 11 17 oxycorticosteroid production throughout life and that during adolescence it commences to produce a second type of ACTH which is responsible for adrenal cortical 17 ketosteroid formation If this thesis is correct one would expect the adrenal cortical zone responsible for 17

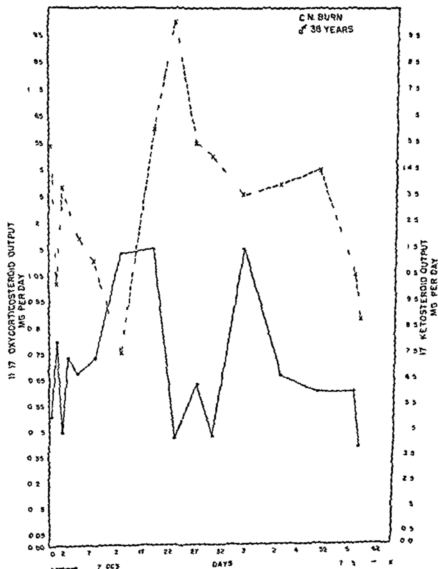


Fig 5 *Urinary 11 17 oxycorticosteroid and 17 ketosteroid excretion by an adult during the first 58 days following a severe burn* Results expressed as mg per 24 hours

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DISCUSSION

DR ROBERT KLEIN I think that there is one note of caution that must be uttered that does not negate Dr Talbot s thesis entirely

We must look with suspicion on any comparison of the relative changes in steroid output in children In the minute amount of chemically measured 17 ketosteroids that young children excrete there is probably little or no androgenically active material The increase in 17 ketosteroids in the urine after ACTH presumably does not represent these nonspecific chromogens but adrenal hormonal products Thus an infinite rise in the output of active substances may be obscured by this error in measurement

DR EDGAR S GORDON (Wisconsin General Hospital and University of Wisconsin Medical School Madison) All of us who have been doing steroid work have run across the phenomenon I am sure in which using the same ACTH preparation in different people gives a vast discrepancy between the 11 oxy and the 17 ketosteroid responses

For example we have many patients who have enormous 11 oxysteroid responses and no rise or no appreciable rise in the 17

of these patients there was a distinct tendency for the 17 ketosteroid and 11 17 oxycorticosteroid output values to undergo opposite variations These changes are in sharp contrast to those seen following exogenous ACTH administration (Figures 1 to 4) where the output of both types of steroid tended to rise simultaneously

Comments Gross divergences in urinary 11 17 oxycorticosteroid and 17 ketosteroid excretion of the types shown in Figures 5 and 6 have not been noted following either the institution or cessation of ACTH treatment Hence it appears unlikely that they can be accounted for by fluctuations in the intensity of stimulus provided by a single ACTH Rather it seems likely that they reflect variations in the concentrations of the two postulated human ACTH s one being concerned with 11 17 oxycorticosteroid the other with 17 ketosteroid precursor production by the adrenal cortex Since the effect of ACTH treatment upon these patients was not studied there remains the remote alternative possibility that the divergences seen were due (a) to changes in the responsiveness of the adrenal cortices to a single ACTH or (b) to transient alterations in the intermediary metabolism of adrenal cortical hormones

SUMMARY AND CONCLUSIONS

Armour ACTH usually causes a simultaneous increase in the output both of 11 17 oxycorticosteroids and 17 ketosteroids in infants, children and adults The human pituitary on the other hand appears capable of stimulating the formation of 11 17 oxycorticosteroids and 17 ketosteroids at independently variable rates Hence it is postulated (a) that the human pituitary secretes two ACTH s one concerned with 11 17-oxycorticosteroid the other with 17 ketosteroid formation and (b) that Armour ACTH either contains both types of ACTH in relatively constant proportions or a single ACTH with dual activity Armour ACTH appears to be about 3 times as potent with regard to 11 17 oxycorticosteroid production as it is with respect to 17 ketosteroid formation

While variations in the quality of ACTH may account in part for differences in urinary steroid excretion noted in subjects of various ages variations in adrenal cortical responsiveness to ACTH also are important A standard single lot of ACTH was observed to induce a relatively much greater increase in urinary 11 17 oxycorticosteroid and a much smaller increase in urinary 17 ketosteroid formation in infants and preadolescent children than in adults It remains to be determined whether these differences disappear after long continued administration of ACTH

DR J S L BROWNE In regard to the 17 ketosteroid response after injury I would like to point out that Dr Forbes and subsequently Dr Nathanson and Dr Cope and we ourselves used to find particularly in burns that there was a reciprocal relation to the corticoids glycogenic corticoids rising and 17 ketosteroids falling However recently as we have improved the nutritional status of our burn cases in our group we have found that the 17 ketosteroids do not fall nearly *so markedly as they used to*

Therefore there is the possibility that either the adrenal response has been modified by the nutritional state or that the subsequent metabolic pathway of the substances has been modified by the nutritional state

DR PETER H FORSHAM It is well established that cortisone and for that matter Compound F will lead to a rise in urinary ketosteroids Therefore if these children tend to put out a lot of 11 17-oxysteroids they eventually will show a rise in 17 ketosteroids

The rise in 17 ketosteroids being preceded by that of the 11-oxysteroids which Dr Browne just discussed probably represents a breakdown of some excess of 11 17-oxysteroids to 17 ketosteroids

Inasmuch as this very small molecule of Lesh s that I spoke about this morning does everything that the large molecule does I would feel that the evidence for the existence of two different types of ACTH while a tantalizing idea has as yet to be established

DR JANET MCARTHUR (Massachusetts General Hospital and Harvard Medical School Boston) Dr Talbot and his associates have made observations which reveal that healthy persons of all ages show an approximately equal increase in urinary 17 ketosteroid excretion following the intramuscular administration of standard doses of testosterone propionate This finding suggests that the infant s capacity to transform and excrete this type of urinary 17 ketosteroid precursor is not greatly different from that of older individuals It follows that preadolescent children should have an appreciable urinary 17 ketosteroid output if they were producing important quantities of these steroids

The essence of all the reservations which have been expressed concerning Dr Talbot s dual ACTH hypothesis seems to be that his is a possible but not a necessary conclusion from the data he has accumulated With the justice of this view Dr Talbot would I am sure be the first to agree His purpose in presenting this material was to provoke discussion and in this undertaking I should say he had notably succeeded!

ketosteroids In the next patient receiving exactly the same preparation he will have an enormous rise in both

This would tend to indicate that it is the adrenal gland itself that is responding differently

DR BRAM ROSE By an unfortunate coincidence I don t happen to have the slide I would like to show that the adrenal may respond in the same patient in different ways depending upon the state of that particular patient

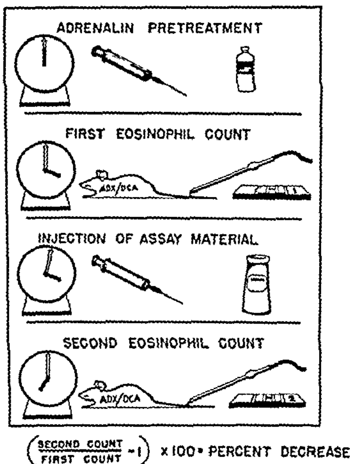
This concerns a patient with lupus whom we have observed for a period of one year She was started on 100 mgs of ACTH in divided doses over a ten day period and then with a lapse of one week she had a second course With the first course the 17 ketosteroids rose quite markedly With the second course they rose to about half that level and with the third course they did not rise at all It was the same patient with the same adrenals

DR JEROME W CONN I would like to inject one more theoretical consideration into this discussion The spontaneous release of endogenous ACTH as the result of stress is not necessarily akin to an injection of exogenous ACTH As we learn more about mechanisms which release ACTH from the pituitary gland endogenously we realize that that total reaction may not represent simply a release of ACTH but a release of some other substances from the pituitary simultaneously as the result of a given stressing situation and that such substances may modify the action of endogenous ACTH as compared with the injected material

We know for example that an injection of epinephrine releases both TSH (pituitary thyroid stimulating hormone) and ACTH at the same time Thus endogenous release of ACTH may involve many other factors besides what we are dealing with when we give exogenous ACTH

DR M M HOFFMAN (Dalhousie University Medical School Halifax) In support of Dr Conn s statement I should like to comment about a patient with rheumatoid arthritis in whom the eosinophile count reached a constant level of approximately 100 per cu mm while receiving cortisone The administration of 100 mgs of ACTH over a 24 hour period did not result in a further depression of the eosinophile count However when the patient experienced severe renal colic the eosinophile count fell to 0 within 8 hours of its onset This suggests that in this individual stress was effective in causing adrenal stimulation under circumstances where exogenous ACTH was without such action

PROCEDURE FOR 7 HOUR BIOASSAY OF ADRENAL CORTICAL HORMONES



$$\left(\frac{\text{SECOND COUNT}}{\text{FIRST COUNT}} - 1\right) \times 100 = \text{PERCENT DECREASE}$$

FIG 1

arithmically It is apparent that the percent decrease of eosinophils during this period is correlated with the dose of hormone injected As little as ½ a microgram of cortisone acetate produced a noticeable decrease and there is practically a straight line dose response between ½ and 6 micrograms

A water soluble cortisone (Free alcohol Merck) produced essentially the same decrease and slope of the dose response curve as the oil soluble acetate Compound F and Compound F acetate likewise produced a response which was essentially identical to cortisone acetate

We have injected many different types of sex hormones oils and non specific steroids and to date the only materials which produce the eosinopenic response in pretreated adrenalectomized mice are the

4

A Seven Hour Bioassay for Urinary Corticoids*

Robert S Speirs, L Wragg Charles D Bonner and F Homburger

R B JACKSON MEMORIAL LABORATORY BAR HARBOR MAINE AND TUFTS COLLEGE MEDICAL SCHOOL BOSTON

A bioassay for adrenal cortical hormones has been developed based upon a decrease in the number of circulating eosinophils in adrenalectomized mice (Speirs and Meyer 1949 1950 1951) This assay highly sensitive and specific for the 11 oxycorticosteroid hormones is performed as follows

1 Jax C_{57} Brown male mice weighing 20 to 25 grams are adrenalectomized in a one step operation and 15 mgm pellets of 11 desoxycorticosteroid acetate are implanted subcutaneously
2 Three days post operatively the mice receive a subcutaneous injection of 20 micrograms of epinephrine and the material to be assayed is injected 4 hours later (See figure 1)
3 Eosinophil counts are taken immediately prior to and 3 hours following the injection of the assay material The percent decrease *in the number of eosinophils during this period is cor* related with the quantity of 11 oxycorticosteroid hormones injected

The following paper is a report of our efforts to adapt this bioassay for quantitative measurement of corticoid excretion in urine of various patients For these assays a special mouse was developed which was equal to the C_{57} Brown in sensitivity to cortisone but more resistant to toxic materials This mouse was the F_1 hybrid between a C_{57} Brown male and a C_{57} Black female

Figure 2 illustrates the results obtained when synthetic adrenal cortical hormones are assayed On the ordinate is plotted the percent decrease in eosinophils and on the abscissa the dose is arranged log

Support for performing the assays came from the American Cancer Society and the Damon Runyon Memorial Cancer Fund through the Cancer Research and Control Unit of Tufts College Medical School and the Medical Research Foundation of Boston Additional support *was obtained from the Division of Research Grants and* Fellowships of the National Institute of Health U S Public Health Service and from the Roscoe B Jackson Memorial Laboratory The large number of 11 desoxycorticosteroid acetate pellets used in these experiments were kindly furnished by Ciba Pharmaceutical Co

same patients before and after treatment and they were found to contain less than 1 microgram per cc

During our early investigations we attempted to inject 3 different quantities of urine and then determine the slope of the dose response curve However as shown in figure 3 all the dose response curves were essentially of the same slope and it was decided that only one point of the curve need be accurately established From that point an estimate could be made of the amount of cortisone necessary to produce a similar response Figure 4 shows the log-dose response curve of the eosinophils to cortisone acetate An example is also given to il-

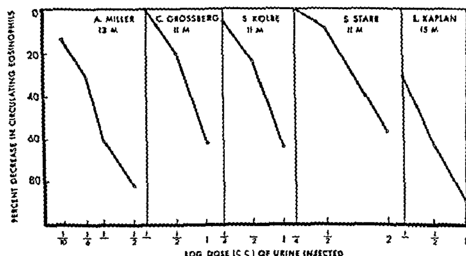


Fig 3

lustrate how we estimate the cortisone equivalent of a urine sample

The next step was to assay urines during a period of time when multiple injections of ACTH were made The results of these assays are shown in Figure 5 The upper part of the figure shows the total volume of urine excreted during a 24 hour period The cortisone equivalent per cc is shown in the middle section and the calculated milligrams of cortisone (equivalent) excreted per day is shown below The level before treatment is illustrated in the first column It can be seen that the quantity of eosinopenic material in the urine increased markedly during the ACTH treatment

The rate of corticoid excretion over shorter periods of time was also determined To do this 2 hour urine specimens were taken before and following an ACTH injection The results are shown in Figure 6

11 oxycorticosteroids of the adrenal cortex (Speirs and Meyer 1951)

Thus in our laboratories we have found the above procedures to be an extremely sensitive and specific test which will quantitatively assay adrenal cortical steroids *It is sensitive to one part per million* of the hormones mixed in water or oil solutions

The next step to consider is whether this test can be used to measure *adrenal hormones* in biological materials such as urine and blood The remainder of this report concerns the results obtained in our attempt to assay the corticoid activity in urine of patients treated with ACTH

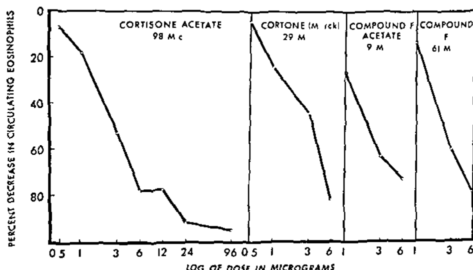


Fig 2

Figure 3 shows the responses obtained when various urines were injected into adrenalectomized mice Again the percent decrease is plotted on the ordinate and the quantity of urine on the abscissa All the urines reported here are from patients who have undergone extensive ACTH therapy

It can be seen that in all cases as little as ½ cc of untreated urine produced a marked decrease in the eosinophils of the experimental animals One half cc of urine obtained from A Miller produced a *decrease of over 80% in the circulating eosinophils during the 3 hour* assay period Even as little as $\frac{1}{10}$ cc produced an eosinopenic response We estimated that this urine contained equivalent to 14 micrograms of cortisone per cc *Control urines were taken from these*

1 The urinary corticoids were relatively high during ACTH treatment Many patients had from 10 to 14 micrograms of cortisone (equivalent) per cc of urine and as much as 21 milligrams equivalent of cortisone excretion per day

2 The corticoid excretion before and after the ACTH treatment was low in the patients observed In most cases there was 1 microgram or less of cortisone (equivalent) per cc of urine or a total daily excre tion of less than 3 milligrams

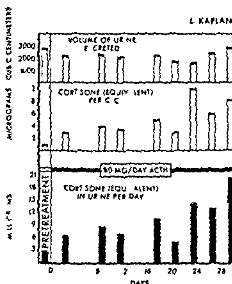


Fig 5

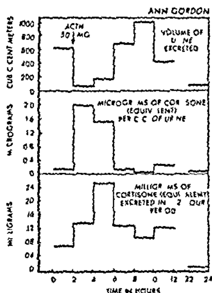


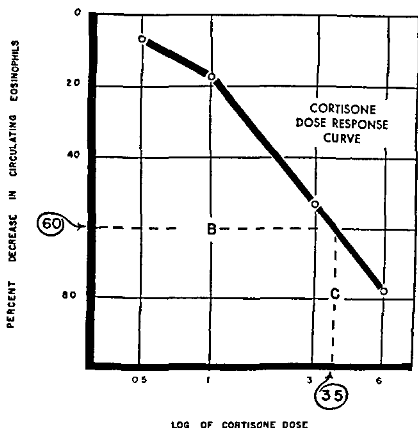
Fig 6

3 Following a single injection of ACTH there was a marked de crease in urine volume lasting for 4 hours During this period there was a marked increase in the quantity of corticoids excreted

4 When urine was treated prior to injection into the assay ani mals it was found that

a) Boiling in a neutral or slightly alkaline medium produced an increase in the assay results This did not occur in urines from patients receiving cortisone treatment
b) Boiling in an acid medium (pH 2) tended to destroy the corti coid activity
c) Concentration of the corticoids could be brought about by evaporating off the water or by extraction with chloroform or ethylene chloride

In summary the eosinopenic response of pretreated adrenalec



TO COMPUTE CORTISONE (Equivalent) PRESENT

A Det m e Average Percent Decre se Produced By M terial Assayed

EXAMPLE ½ cc of urine injected to e ch of 6 M ce p oduced the follow ng perce t decreases 54 46 59 57 74 and 68 The a ge perc nt decrease was 60

B Locate Percent Dec ease On Establ shed Cort sone Dose Respo se Curve

See Chart

C On Abscissa Read Off Dose Of Cort o e Which P oduces Th t Response

EXAMPLE: 60% d c ease s equ valent to 3 5 M crogr m of Cort Thus 1 cc wo ld conta n tw ce as m ch o b qu al t to 7 0 M c og ams of Cort so e

Fig 4

It can be seen that there was a tremendous decrease in the quantity of urine excreted for a period of 4 hours following the ACTH injection During this period of depressed excretion there was a marked increase in the quantity of corticoids per cc of urine

During the course of our experiments the following observations were made

an enormous discrepancy Possibly the fact that Speirs assay uses urine without any extraction has a terrific advantage because when you extract urine for corticosteroids some as you all know are undoubtedly destroyed by the usual extraction procedure

DR ROBERT S SPEIRS Thank you Dr Lewis for your comments It is certainly very encouraging to know that other laboratories are able to substantiate our findings

Dr Pincus is entirely right We have been finding a much greater quantity of corticoid material in the urine than that reported in the literature by Dr Venning and others In some cases we have found up to 20 milligrams equivalent of cortisone excreted per day These high values have bothered us for a long while and we have attempted to determine whether our assay is specific or whether some augmenter might be misleading us All evidence to date strongly indicates that the assay is specific at least as far as the steroids and the more commonly available hormones are concerned Over fifty steroid materials have been assayed and only adrenal cortical materials have shown any eosinopenic activity in pretreated adrenalectomized mice

I would like to point out that we have had a tremendous advantage in our work Because the eosinopenic response is extremely sensitive we were able to assay many urines without having to first concentrate the active material A comparison of assays made before and after treatment helped us to evaluate the effectiveness of the many methods of concentrating the corticoids

In the above manner we have performed chloroform and ethylene chloride extractions of urine and found that the eosinopenic material was extractable It was also readily soluble in 10% alcohol However the chloroform or ethylene chloride did not remove all the active material a small amount usually less than 1/3 remained in the urine

The eosinopenic material can be destroyed by boiling under acid conditions (pH 2) This agrees with published reports of Dr Pincus and others However we have found that if the urine is boiled under neutral or alkaline (pH 10) conditions an increase in activity occurs In some of our experiments the activity of a sample doubled after boiling (without loss of volume) for 20 to 40 minutes This increase only occurred in patients receiving ACTH and did not occur in patients receiving cortisone therapy

As far as augmenters are concerned we have not been able to detect any marked augmenting effect when known amounts of water soluble cortisone were added to various urines

Thus in our laboratory the eosinopenic assay of corticoid appears to be specific sensitive and accurate

tomized mice can be used as a sensitive method for assaying urinary corticoids

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DISCUSSION

DR ROGER A LEWIS (Hoffman La Roche Nutley) I am interested in this paper and I would like to mention that we have done a few experiments of the same general type based upon the previous work of Speirs and Meyer which appeared in Endocrinology We found much less active material than the present group claims

Our work also utilized adrenalectomized mice maintained with pellets of desoxycorticosterone and we relied very heavily on Dr Venning s previous work for guidance By measuring the eosinophil counts we found urines to contain approximately the same cortisone or Compound E equivalent as Dr Venning found when she assayed for glycogenic material

This method has been described in the current number of the Journal of Applied Physiology and the only interesting point which I would like to make is that we find the same results when we measure eosinopenic activity as when Dr Venning measures glycogenic activity This holds true with all the compounds we have tested and it holds true with all the urine extracts that we both tested

DR GREGORY PINCUS I am interested in Dr Lewis comment because I thought I had read Dr Speirs tables incorrectly

Dr Lewis says that he gets about the same activity as the glycogenic unitage described by Dr Venning but I notice in Speirs table 3 mg equivalent per day of cortisone in contrast to Venning s 20 to 40 micrograms a day of Compound E equivalent That is certainly

an enormous discrepancy Possibly the fact that Speirs assay uses urine without any extraction has a terrific advantage because when you extract urine for corticosteroids some as you all know are undoubtedly destroyed by the usual extraction procedure

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5

The Effect of ACTH upon Steroidogenesis by the Isolated Perfused Adrenal Gland*

Gregory Pincus, Oscar Hechter and A Zaffaroni†

WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY SHREWSBURY MASSACHUSETTS AND ROCHESTER UNIVERSITY MEDICAL SCHOOL ROCHESTER

INTRODUCTION

It scarcely seems necessary to emphasize to this audience the importance of characterizing the exact nature of the adrenal cortex secretion The most direct approach to this problem is obviously to analyze the steroids released from intact adrenals but until recently this has not been undertaken Two principal methods have been employed the first involving the removal of adrenal venous blood from living animals[1] the second utilizing isolated glands perfused with homologous blood From both types of preparations it has been observed that the adrenal venous blood following ACTH contains 17 hydroxycorticosterone corticosterone and possibly other steroids[3,4] Today we should like to present a more complete characterization of the steroidal products released by the perfused beef gland which demonstrates that the adrenal cortical secretion may represent a complex mixture of corticosteroids In addition we wish briefly to report tracer studies which conclusively demonstrate that corticosteroids can be formed from radioacetate

METHODS

In these studies we have employed beef adrenal glands mounted in a perfusion apparatus previously described[5] The medium used was citrated homologous whole blood In each experiment a period of perfusion of the gland by blood without added ACTH was followed by a similar period of perfusion with blood containing ACTH

* These investigations were aided by grants in aid from G D Searle & Co and the U S Public Health Service (RG 999)

† Post doctoral Fellow of the National Cancer Institute

The ACTH employed was an Armour preparation and was used in a concentration of approximately 6 mg LA 1A equivalent per liter of blood The extraction of steroids from the blood on a charcoal adsorbate as well as the elution and concentration of the crude steroid mixture has been previously described[6] the crude concentrate obtained was analyzed by paper chromatography using the systems developed by Zaffaroni et al [7,8]

RESULTS

THE α KETOLS OF BEEF BLOOD

Preliminary analysis disclosed the presence of at least fifteen α ketols in the steroid mixture obtained from ACTH stimulated glands (cf Tables I to III) An analysis of several 2 liter samples of beef blood which had not been perfused through adrenals demonstrates the presence of some of these substances but the variability from sample to sample was quite large one sample having a total concentration of 0 6 mg per liter of identifiable α ketol components whereas another had only 0 1 mg per liter In all of these blood samples analyzed 17 hydroxycorticosterone was consistently present (in amounts ranging from 35 to 180 micrograms per liter) and corticosterone 11 desoxycorticosterone and certain highly polar compounds (Unknowns I to V) were usually identifiable

SINGLE CYCLE PERFUSIONS

In a number of experiments aliquots were taken from a large single pool of beef blood for analysis (a) for the initial steroid content (b) after adrenal perfusion without added ACTH and (c) after adrenal perfusion with added ACTH The blood was circulated through the gland and collected without recirculation Table I contains the data of such a single-cycle experiment The data demonstrate that after passage through the gland in the absence of ACTH the blood contains all of the identifiable steroid components albeit in rather low concentrations In this instance the total corticosteroid content after perfusion without ACTH is less than the original blood concentration suggesting uptake of blood steroids by the perfused tissues In other experiments however a significant but small net release of steroids occurs after adrenal perfusion in the absence of ACTH Independent of whether there is a net uptake or release corticosterone and 17 hydroxycorticosterone are the principal single components of such adrenal perfusates Following ACTH the adrenal perfusates show consistently a high concentration of all components In this particular experiment one observes a six fold increase of total corticos

teroid over that obtained from the gland without ACTH perfusion with individual components increasing 3 to 9 fold

The consistency of the steroid pattern observed following ACTH is illustrated by the data of Table II in which are presented the data of four separate single-cycle experiments In these as in the previous experiment (Table I) 17 hydroxycorticosterone and corticosterone

Table I

THE α KETOLS PRESENT IN ADRENAL PERFUSATES

α Ketols	Micrograms of Steroid per 2 Liter Blood Sample		
	Not Perfused through Adrenal	Adrenal No ACTH	Perfusate ACTH
Unknowns I–V	220	80	700
17 Hydroxycorticosterone	360	145	1100
Cortisone	—	25	200
Unknown VI	—	40	
Unknowns VII–IX	110	45	250
Corticosterone	400	230	1100
Unknown X	—	60	300
Dehydrocorticosterone	—		250
Desoxycorticosterone	120	35	140

are present in approximately equal amount and represent between them 54% to 61% of the total α ketol In all but one instance the highly polar unknowns I to V are the next largest component (10% to 17% of the total) the complex of unknown X plus 11 dehydrocorticosterone and that of unknowns VII to IX are next in amount and in lowest concentrations usually are the complex of cortisone plus unknown VI and 11 desoxycorticosterone

The corticosteroids thus far clearly identified as being present are 17 hydroxycorticosterone corticosterone 11 dehydrocorticosterone 11 desoxycorticosterone and cortisone Of the ten substances labelled as unknowns we have evidence that among them are allopregnanetetrol 3(β) 11 17 21 one 20 or Reichstein s compound V (unknown III) allopregnanetriol 3(β) 11 17 one 20 or Reichstein s compound P (unknown VI) and allopregnanediol 3(β) 21 one 20 (unknown X) It should be noted that among the 11 oxygenated compounds identified the 11 hydroxylated steroids predominate the 11 ketones representing a much smaller quantity and the 11 desoxy substances representing the least It should be noted that Reichstein s S

Table II

MICROGRAMS (PER 2 LITERS) OF VARIOUS α KETOLS IN THE PERFUSED BLOOD AFTER THE PERFUSION OF ACTH (6 MG PER LITER) AT A FLOW OF 1 LITER PER HOUR

Experiment	*1*	*2*	*3*	*4*
*α Ketols**				
Unknowns I-V	450	600	550	700
17 OH Corticosterone	1000	1100	1700	1100
Cortisone	120	} 60	50	} 200
Unknown VI	120		110	
Unknowns VII-IX	150	—	800	250
Corticosterone	1200	1300	1800	1100
Unknown X	} 450	} 400	330	300
11 Dehydrocorticosterone			330	250
11 Desoxycorticosterone	120	140	—	—

Unknown III may be allopregnantetrol 3β 11 17 21 one 20
Unknown X may be allopregnanediol 3β 21 one 20
Unknown VI may be allopregnanetriol 3β 17 21 one 20

17 hydroxydesoxycorticosterone does not appear in our listing of steroid ketols present in perfusates although our methods should have revealed it if present in greater than 10–20 micrograms

MULTIPLE CYCLE EXPERIMENTS

The data of the single cycle experiments (Tables I and II) represent adrenal products issuing from the gland with no possible further action of the gland tissue upon them In additional experiments we have recirculated the perfusion medium through the gland a number of times and have examined the products obtained Banks of five glands were perfused with ACTH for four successive hours and in that four hour period the flow rate was varied to give varying numbers of passages of the entire perfusate through the glands In Table III we present the data of three separate experiments in which the adjusted flow rates allowed 35 47 and 56 cycles of the medium in the four hour period of perfusion While the total steroid outputs of experiments 1 and 3 approximate each other a much lower total output was obtained in experiment 2 This we interpret as due to adventitious differences in the glands themselves in any event no consistent effect of recycling on total output is observable In each experiment 17 hydroxycorticosterone is the largest single product representing 40% to a little over 50% of the total corticosteroid Although corticosterone is the next most plentiful component the corticosterone 17 hydroxycorticosterone ratio is now not 1 1 (as in single cycle experiments) but varies from 1 2 3 to 1 3 5 suggesting that the synthesis of 17 hydroxycorticosterone is differentially promoted by the recy

teroid over that obtained from the gland without ACTH perfusion with individual components increasing 3 to 9 fold

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each as isolated is practically identical (290 counts per mg per minute for corticosterone 310 counts per mg per minute for 17 hydroxy corticosterone with a thin window counter) suggesting that these compounds arise from a common precursor

CONCLUSIONS

The data of these experiments seem to us to demonstrate unequivocally that ACTH acting directly on the adrenal gland stimulates the production of a multiplicity of corticoid α ketols Furthermore the synthesis of significant amounts of these substances appears to depend on the presence of ACTH in the circulating medium since the perfusate without ACTH is scarcely enriched with additional corticosteroid Although the major products of perfusion are corticosterone and 17 hydroxycorticosterone the presence of thirteen additional α ketols suggests the genesis of multiple products some of which may be precursors while others are further derivatives of corticosterone and 17 hydroxycorticosterone The fact that 11-desoxycorticosterone increases significantly after perfusion with ACTH taken together with our previous demonstration[49] that this steroid is converted to corticosterone by an adrenal enzyme suggests this compound as a likely precursor of corticosterone

While clearly a precursor 11 desoxycorticosterone appears from these data also to be an adrenal secretory product The status of this compound as an adrenal steroid has long been in doubt because it was obtained only in minute amount from adrenal tissue only by Reichstein et al [10] Its powerful electrolyte activity and the peculiar pathological consequences of overdosage with desoxycorticosterone suggest important consequences of its endogenous release

Until full identification of the various perfusion products is achieved the nature of their interrelations cannot be surmised Nonetheless it is interesting to note that Reichstein and coworkers[10] have isolated 12 α ketols from adrenal glands taken at slaughter including his substance S which has not as yet been observed in adrenal perfusates This means that we have obtained at least four hitherto unrecognized adrenosteroids in these perfusates Perhaps the so called amorphous fraction of adrenal extracts represent in part a mixture of our unknowns I to X It should be emphasized that our analyses measure only the ketols present so that other types of steroid (e g estrogen androgen progestin) obtained by adrenal extraction may well be present Experiments now in progress have as their special aim the search for such non corticoid steroids

The fact that acetate is a corticosteroid precursor has been firmly established by our findings In view of the fact that acetate is also a

cling We do not believe that conversion of corticosterone to 17 hydroxycorticosterone occurs since our previous experiments[6] with the perfusion of corticosterone through the gland gave no indication of such conversion

In Table III we compare the mean output per 20 gm of gland per hour with the observed corticosteroid content of beef adrenal glands[8] These data demonstrate conclusively the remarkable corticosteroidogenic activity of ACTH perfused glands in one hour under these perfusion conditions a 20 gram gland puts out approximately nine times the observed corticosteroid content and furthermore this rate of corticosteroidogenesis is maintained over several hours The greatest relative increase occurs in the case of 17 hydroxy corticosterone (almost 30 fold) suggesting that this substance is a major end product of corticosteroidogenesis

Table III

MULTIPLE CYCLING OF THE PERFUSED ADRENAL AND α KETOL PRODUCTION (IN MICROGRAMS)

Experiment No and Cycle No	*1–35*	*2–47*	*3–56*	*Mean Production per 20 gm of Gland per Hour*	*Micrograms per 20 gm of Beef Adrenal*
α Ketols					
Unknowns I–V	1390	445	830	205	70
17 OH Corticosterone	6250	1330	6400	1155	40
Cortisone / Unknown VI	1160	665	690	205	20
Unknown VII	210	80	195	40	25
VIII	290	45	195	43	
IX	290	80	280	52	
Corticosterone	1850	450	2800	408	70
Unknown X	—	90	208	33	35
11 Dehydrocorticosterone	580	225	555	105	
11 Desoxycorticosterone	—	—	140	38	?

THE PERFUSION OF ADRENAL GLANDS WITH RADIOACETATE (CH_3C^*OOH)

In several experiments we have added to the ACTH containing perfusion medium about ½ mc of C 14 carboxyl labelled sodium acetate The steroidal products are still being analyzed but to date from two such experiments corticosterone and 17 hydroxycorticosterone have been isolated and identified The radioactive count of

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DISCUSSION

DR KONRAD DOBRINER I would like to ask Dr Pincus if his results indicate that Compound E is already a metabolite and is not a true adrenal hormone in the sense that Compound F and Corticosterone are I was impressed by the fact that there was very little Compound E and 11 dehydrocorticosterone in comparison with the 11 hydroxy compounds in the perfusate This would surely suggest that the 11 ketones were secondary products and not the hormones

DR LEONARD P ELIEL Dr Pincus raised the possibility that the effects seen from stimulation of the adrenal cortex by ACTH might be different from those seen with administration of the pure steroids by virtue of the fact that many different steroids were identified

We have studied two patients with chronic lymphatic leukemia in each of whom two balance studies were done—one with cortisone and one with ACTH—and we compared the metabolic effects of the two

In so far as the clinical and hematologic effects and the effects on nitrogen and electrolyte metabolism were concerned there were no essential differences between cortisone and ACTH

There was some water retention seen with ACTH which might be attributed to an antidiuretic substance and which was not seen following administration of cortisone

DR JEROME W CONN I would like to ask Dr Pincus if he has any ideas about the difference between a single circulation in which corticosterone and 17 hydroxycorticosterone appeared to be produced in about the same quantity while on multiple circulations there was a marked increase in favor of Compound F over Compound B

DR GREGORY PINCUS In regard to Dr Dobriner s question the data would suggest that Compound E is a metabolite of Compound F We are inclined to believe that this is true because in addition to the secretory tissue that this blood circulates through there is also a good

precursor of adrenal cholesterol[11] which is generally regarded as the precursor of steroid hormones[1] our findings are perhaps not too surprising We have perfused C 14 labelled cholesterol through the isolated beef adrenal gland and are now in the process of examining the products from the data of these experiments we hope to be able to determine whether acetate goes directly to corticosteroid or if it is first built up into cholesterol which is then secondarily degraded to corticosteroid

Finally we should like to mention briefly some of the implications of our data for clinical practise Our finding that ACTH stimulation leads to the production of a multiplicity of products suggests that ACTH therapy may lead to consequences quite different from therapy with cortisone or any other single corticosteroid Not only must the special effects of the known active substances be reckoned with but the possibility must be considered that components which we designate as unknowns I X likewise may possess biological activity In addition possible synergisms and/or antagonisms between individual components of this complex may be discovered While the pattern of secretion observed with normal beef adrenals is remarkably consistent this uniformity might very well be altered in pathological glands so that the steroid mixture released no longer contains 17 hydroxycorticosterone and corticosterone as the major components

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6

The Cortical Steroid in Mammalian Blood After ACTH Stimulation

Don H Nelson Leo T Samuels and Hans Reich
UNIVERSITY OF UTAH COLLEGE OF MEDICINE, SALT LAKE CITY

Since the isolation of the adrenal steroids by Kendall Reichstein and coworkers there has been some question as to which of these 28 or more compounds are actually secreted by the adrenal gland Vogt Pashkis and others have shown that adrenal cortical activity as demonstrated by biological assay is present in adrenal venous blood Vogt was able to find little or no adrenal activity in peripheral blood

We have recently been interested in isolating the steroids which are present in adrenal venous blood This work is still in progress but we now have evidence for the presence of a number of compounds in this blood

Perhaps I should describe briefly the manner in which this adrenal blood has been collected The left lumbo adrenal vein of the dog has been cannulated following the operation outlined by Vogt and approximately 30–100 ml samples of blood collected continuously over a period of 1–3 hours In some cases after obtaining a control sample 5 mgm of ACTH has been given intravenously Using a chromatographic method which we have developed for the measurement of adrenal steroids in blood we have been able to demonstrate quantitative changes in adrenal steroid secretion following ACTH as is illustrated in the next two figures

Figure 1 represents the findings when no ACTH was given Figure 2 shows the immediate marked increase in steroid secretion when ACTH is given Despite the stress of the operation and the obvious assumption that these animals pituitaries were secreting ACTH the steroid levels have remained remarkably constant when no ACTH was administered An increase in blood flow from the cannula has been observed immediately following the intravenous ACTH The mechanism by which this is produced is obscure at this time

Table I illustrates the pattern of secretion of adrenal steroids as

deal of non specific tissue in our perfusion preparation There is the adrenal medulla for one the arteries and veins and similar tissue so the suggestion that cortisone is a metabolite of Compound F I think is acceptable

In regard to Dr Eliel s comment, we consider cortisone an end product and that Compound F is its precursor I should like to know what experience he had with Compound F I suspect it was different

In regard to Dr Conn s question we have an explanation Originally we thought that corticosterone might be converted by the adrenal to Compound F but we tested this in actual experiments and found that it did not occur Corticosterone appears unchanged after perfusions through the adrenal

What we are now inclined to believe is that in some way Compound F inhibits those enzymatic processes which lead to the production of Compound B In other data we have presented we have suggested that in the adrenal there are two paths of synthesis one leading to the 17 hydroxylated type of steroid of which Compound F is the example and the other to the 17 desoxy type It may be there is competition for substrate between these two systems and the presence of Compound F in some way inhibits corticosterone synthesis We hope to prove this by the simultaneous perfusion of the precursors of each which we already know

lations from dog adrenal vein blood Compound F (17 hydroxycorticosterone) predominates following ACTH administration Further work is under way to more definitely establish relative quantities of these substances present under various conditions The third fraction is eluted with 25% ethanol in $CHCl_3$ and is inconsistently present This compound has the $\alpha\beta$ unsaturated structure as evidenced by ultraviolet absorption but it has not been definitely characterized

Other investigators working independently have confirmed the presence of some of these steroids in the adrenal effluent 17 hydroxy corticosterone and corticosterone have been identified by Zaffaroni and 17 hydroxycorticosterone by Bush

Our evidence agrees with Vogt s findings that the adrenal gland is able to secrete very large quantities of adrenal steroids Under the

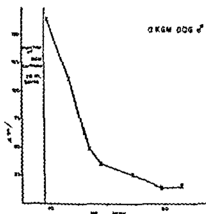


Fig 3

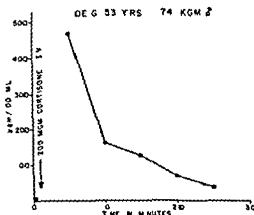


Fig 4

conditions of our experiment a 10 kgm dog would secrete approximately 50 mgm of steroid per day Despite these very large amounts secreted only a small amount of free adrenal steroid can be measured in peripheral blood Only about 5 micrograms per 100 milliliters of the 17 21-dihydroxy 20 ketone compound has been found in peripheral human venous blood

Preliminary experiments conducted in both the dog and man demonstrate rapid removal of intravenously injected cortisone from the blood stream as is illustrated in figures 3 and 4

With the cooperation of Dr Hans Hecht of the Department of Medicine we have been able to cannulate the renal vein of human subjects Dr Hecht has made use of the anatomical fact that the adrenal vein often empties into the left renal vein We might therefore expect to find higher levels of adrenal steroids in renal blood than we find in peripheral blood Figure 5 demonstrates that this is so and

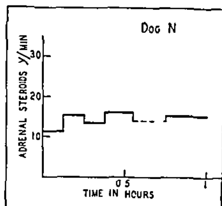


Fig 1

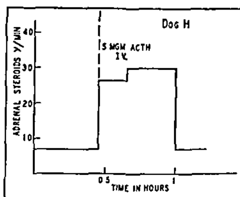


Fig 2

Table I

Fractions	*Fractions in Which Pure Steroids Are Eluted*	*Relative Amounts of α β Unsat Found in Adrenal Vein Blood*	
$CHCl_3$ (last 12 ml of 50 ml)	Testosterone	±	
2% ethanol in $CHCl_3$ (12 ml)	Testosterone Progesterone Androstenedione	++	
ditto	ditto	+	
10% ethanol in $CHCl_3$ (12 ml)	E Corticosterone F DOC	++++++++++	A
ditto	ditto	+++++	A
ditto	ditto	++	A
ditto	ditto	+	A
15% ethanol in $CHCl_3$ (12 ml)		±	
25% ethanol in $CHCl_3$ (12 ml)		++	
ditto		+	
ditto		+	
Absolute ethanol (12 ml)		±	

Table 1 Elution pattern of extract from adrenal vein blood and of various pure steroids
A 17 hydroxycorticosterone and corticosterone have been identified in this fraction (Nelson Reich Samuels *1950*) (Reich Nelson Zaffaroni *1950*)

demonstrated by our method It can be seen from this slide that chiefly 3 fractions have been obtained The first which is eluted from the column by 2% ethanol in $CHCl_3$ comprises compounds which are less polar than the adrenal steroids 17 hydroxycorticosterone and corticosterone have been identified in the second fraction In our iso

lations from dog adrenal vein blood Compound F (17 hydroxycorticosterone) predominates following ACTH administration Further work is under way to more definitely establish relative quantities of these substances present under various conditions The third fraction is eluted with 25% ethanol in $CHCl_3$ and is inconsistently present This compound has the α β unsaturated structure as evidenced by ultraviolet absorption but it has not been definitely characterized

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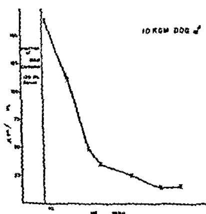


Fig 3

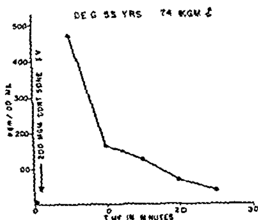


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that an increase in adrenal steroid secretion after ACTH administration can be demonstrated by this method

CONCLUSION

In conclusion we may say that the adrenal gland secretes corticosterone 17 hydroxycorticosterone and at least two other compounds Under conditions of stress when stimulated by ACTH the chief steroid secreted appears to be 17 hydroxycorticosterone The

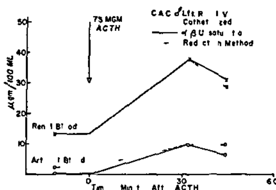


Fig 5

very low level of cortical steroids in peripheral blood is apparently due to rapid removal of these compounds from the circulation

DISCUSSION

DR KARL F PASCHKIS (Jefferson Hospital and Jefferson Medical College of Philadelphia Philadelphia) We have been conducting experiments on dogs which confirm the observation of the Utah group concerning the rapid disappearance We have not used cortisone intravenously but Upjohn s aqueous extract and our experiments were designed for a slightly different purpose

We are comparing the clearance from the peripheral blood in normal dogs and in dogs under stress trying to get some idea or some approach to the mysterious question of utilization of adrenal hormones

Our method of assay is Venning s bioassay We have found so far that in the intact dog the clearance rate under stress differs little from that under normal conditions As stressing agents we have used insulin or colchicine In adrenalectomized dogs there is perhaps a more rapid clearance but again with no appreciable difference between stressed and non stressed animals

DR DON H NELSON I would like to make one point that I don't think I made clear When ACTH is given both the 2 per cent fractions containing the less polar substance and the 10 per cent fraction from which corticosterone and 17 hydroxycorticosterone have been identified are increased

7

The Effect of ACTH on the Urinary Excretion of Steroids in Neoplastic Disease* †

Ira T Nathanson Lewis L Engel and Rita M Kelley

COLLIS P HUNTINGTON MEMORIAL HOSPITAL MASSACHUSETTS GENERAL HOSPITAL AND HARVARD MEDICAL SCHOOL BOSTON

The effect of ACTH on the urinary excretion of ketosteroids and adrenal cortical hormone metabolites which retain their reducing and formaldehydogenic properties has been studied in the past However the effect of this hormone on the excretion of non ketonic alcohols and estrogens has not been studied extensively The development of a battery of analytic procedures applicable to single twenty four hour urine specimens has made it possible to follow day by day changes in five groups of steroid compounds in suitable patients

METHODS

Twenty four hour urine specimens were collected and the completeness of the collection controlled by creatinine determinations

Ketosteroids were measured by the Zimmermann reaction using the Nathanson Wilson modification[1] of the Holtorff Koch aqueous alkali method

Total and non ketonic steroid alcohols were estimated by the dinitrophthalate method[2]

Total estrogens were measured by photofluorometry and the individual estrogens separated analyzed and characterized by counter current distribution[3]

Extracts containing the reducing and formaldehydogenic steroids were prepared after hydrolysis of the conjugated steroids with β glucuronidase Reducing activity was measured by the Heard Sobel and Venning method[4] and formaldehydogenic activity by a modification

* This work was supported in part by a grant from the National Cancer Institute U S Public Health Service and in part by an Institutional Grant from the American Cancer Society to the Massachusetts General Hospital

† This is publication No 721 of the Cancer Commission of Harvard University

of the Daughaday Jaffee and Williams procedure[5] which eliminates distillation of the formaldehyde

The present report embodies the results obtained in ten patients with malignant disease who were given ACTH Complete studies were made in six of these patients

The data summarized in Fig 1 indicate that within the narrow range of dosages of ACTH employed (45–100 mg) in the patients under study the rise in one fraction of the urinary steroid mixture is not necessarily associated with simultaneous rises in other fractions Patient N T provides an example of this point The moderate rises in non ketonic alcohol and estrogen excretion were associated with an unaltered ketosteroid level and actual decreases in reducing and formaldehydogenic steroid excretion A similar if not as marked dissociation may be seen in the other patients studied These individual variations make it difficult to decide which if any fraction of the urinary steroids may be useful as an index of ACTH effect Further experiments are required to elucidate these points

It would be interesting to determine in individual patients the dose of ACTH required to cause an arbitrarily determined change in one of the fractions of the urinary steroids and then to examine the relative effects on the other fractions of the urinary steroid mixture Such data might provide a better index of the dosage required to produce a given response and also shed further light on the dissociation of response with respect to the various steroid fractions

The dose range used in these studies was chosen deliberately in the hope of bringing out variations in the responses of the adrenal glands of individual patients to ACTH It was anticipated that this dose level would be critical for the differentiation of more responsive from less responsive glands in patients with some diseases in which remissions might be expected under ACTH therapy The two patients with Cushing s disease do not fall into this category but they do demonstrate that this dose level is adequate to produce a response in a sensitive adrenal cortex

The increased excretion of estrogen by all of the female patients studied to date is noteworthy Neither of the two male patients in the series showed any significant change in estrogen excretion In one of these however (O N) the picture was complicated by the coexistence of post hepatitis cirrhosis and an abnormally high excretion of estrogen during the control period

Sufficient material has been accumulated in patients N T (malignant lymphoma) O D (multiple myeloma) and E B (Cushing s disease) to carry out separations and analyses of the individual estrogens by countercurrent distribution As may be seen from Table I no estrone or estradiol and less than 1 microgram of estriol per day

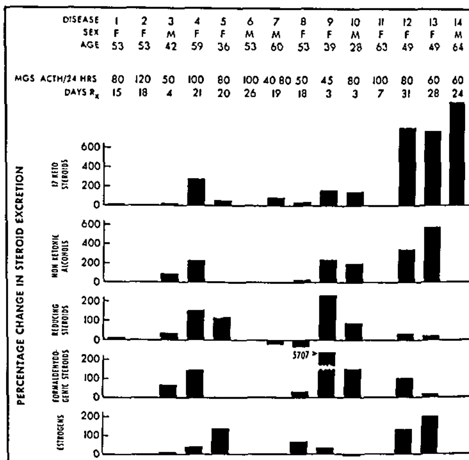


FIG 1 Steroid excretion after ACTH therapy in neoplastic disease The data are plotted as per cent change of the steroid fractions from the pre treatment control level

Code to Diseases

1 and 2	E L R	—Cancer of Breast (same patient at different dose level)
3	A O N	—Cancer of Lung
4	A K	—Chronic Lymphatic Leukemia
5	B H	—Malignant Melanoma
6	J I	—
7	V B	—Malignant Lymphoma
8	N T	—
9	E B	—Cushing s Disease
10	G W	—
11	G H	—Multiple Myeloma
12 and 13	O D C	—Multiple Myeloma (same patient at different dose level)
14	J S	—Neurofibrosarcoma

were excreted during the control period by patient N T although the total estrogen excretion was 72 µg per day This value represents the excretion of fluorogenic phenols which may or may not be steroidal in nature but which are not identical with estrone estradiol or estriol During the period of treatment with ACTH an average daily excretion of 19 µg of estriol was superimposed upon the base line excretion There still remains a discrepancy between the sum of the average daily excretion of 121 µg per day and the basal excretion plus estriol This may be accounted for by an increase in non specific fluorogen excretion or possibly the excretion of steroidal phenols which have not yet been recognized as estrogen metabolites The adrenal origin of the estriol must be considered since at autopsy this

Table I

URINARY EXCRETION OF ESTROGENS AFTER ACTH THERAPY

Name	Age	Sex	Disease	Dose mg × days	Estrogens (µg/day) Tot	E_1	E_2	E_3
N T	53	F	Malig lymph	0	72	0	0	<1
				50 × 9	121	0	0	19
O D	49	F	Mul myeloma	0	25	<2	0	<7
				80 × 19	89	4	3	29
E B	39	F	Cushing's	0	104	12	16	16
				45 × 3	136	27	24	25

Code to estrogens
E_1—Estrone
E_2—Estradiol 17β
E_3—Estriol

patient was found to have atrophic ovaries Patient O D also showed an increased excretion of estrogen during the period of ACTH administration

In the case of patient E B estrone estradiol and estriol were excreted during the control period but there was a significant increase during the treatment period It is interesting that the residual excretion of 60 µg fluorogenic phenols per day seems to remain constant during the control and treatment periods

Examples of excretion patterns of the types of steroid compounds under study are shown in the three figures The patient had multiple myeloma and improved dramatically during treatment The excretion of ketosteroids and steroid alcohols are presented in Fig 2 It will be seen that during the periods of ACTH therapy the excretion of both of these components rose markedly When the dosage was lowered from 80 to 60 mg per day there was a slight fall and a return to

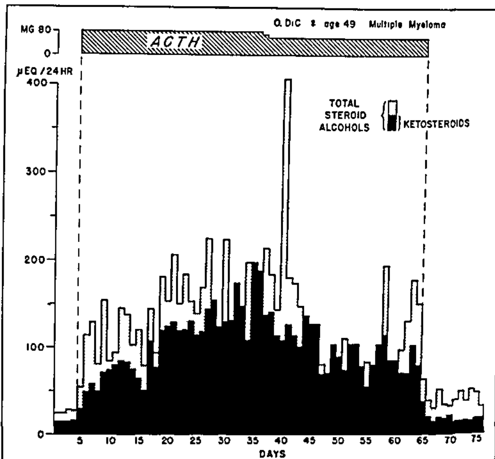


FIG 2 The urinary excretion of ketosteroids and steroid alcohols in a patient with multiple myeloma treated with ACTH Specimens were collected daily but during part of the second period of ACTH therapy alcohol determinations were made every fourth day

the control level upon cessation of therapy The data in Fig 3 show that during the first period of therapy the level of reducing steroid excretion was built up quite rapidly stabilized for a short time then fell and subsequently built up to a stable level somewhat lower than the first plateau The formaldehydogenic steroids also showed a biphasic response which differed from that of the reducing steroids in that the second plateau was nearly twice that of the first When the dosage of ACTH was lowered to 60 mg per day both components maintained a fairly steady but somewhat lower level Cessation of therapy was soon followed by a fall to control levels The estrogen excretion in this patient is shown in Fig 1 In this case there was a slow rise in estrogen excretion to a plateau which was maintained

until the dosage of ACTH was reduced from 80 to 60 mg per day The level of estrogen excretion then fell somewhat and finally returned to the control level when therapy was stopped

It is interesting to note that all of the urinary steroid fractions do not follow the same pattern in time The rise in urinary estrogens appears to be slower than the rises in ketosteroids and alcohols and

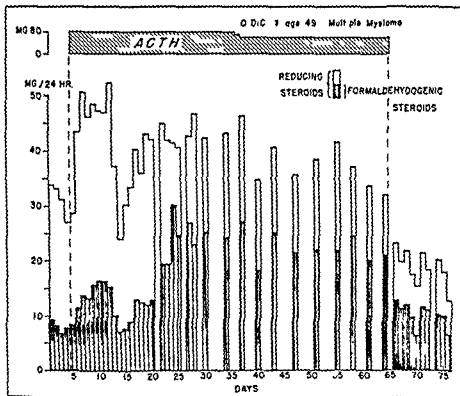


FIG 3 The urinary excretion of reducing and formaldehydogenic steroids in a patient with multiple myeloma treated with ACTH Specimens were collected daily but during the second half of the experiment measurements were made at 3–4 day intervals

the biphasic behavior of the reducing and formaldehydogenic steroids serves to differentiate them from the other groups

It may be postulated that the action of ACTH is exerted early in the chain of reactions leading from cholesterol to the various types of adrenal cortical steroids and in this case the dissociation observed may be ascribed to variations in the metabolic pathways lower down in the chain Larger doses of ACTH may serve either to accentuate or to mask these dissociations Furthermore these variations in metabolic pathways are by no means constant as may be seen from Figs

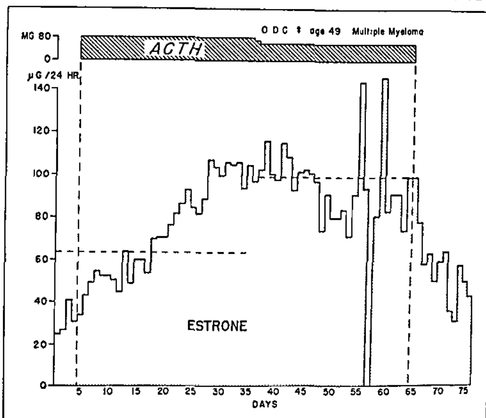


FIG 4 The urinary excretion of estrogens in a patient with multiple myeloma treated with ACTH Specimens were analyzed daily

1–3 As the course of ACTH administration is continued the excretory levels of the various urinary steroid fractions vary considerably These day to day changes may reflect some subtle alterations in metabolic status of the patient

CONCLUSIONS

1 A study of the excretion of ketosteroids steroid alcohols estrogens and reducing and formaldehydogenic steroids in eight patients with neoplastic disease and two patients with Cushing s disease who were treated with ACTH has revealed striking variations in the urinary excretion patterns of these steroids

2 *It is believed that the low dose level employed (45–100 mg per day)* accentuates these variations

3 An increased excretion of urinary estrogens was observed in all six of the female patients in whom these measurements were made Neither of the two males showed this effect

4 The most constant finding was an increased excretion of steroid alcohols during ACTH therapy

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DISCUSSION

DR KARL F PASCHKIS We were interested to see whether ACTH would stimulate pregnanediol and estrogen excretion by the adrenal cortex

Table II and Fig 5 shows the results in three male patients

Table II

Excretion of Pregnanediol and of Estrogens in Men During Treatment with ACTH and Cortisone

Case Number Age Diagnosis	*Treatment*	*Pregnanediol mg per 24 hrs*	*Estrogens International Units per 24 hrs*
1 30 yrs Chorioiditis	ACTH 40–100 mg daily	3 2 3 9 18 5 17 5 3 0	
2 44 yrs Uveitis	ACTH 100 mg daily	8 0	1000
3 34 yrs Periart Nod	ACTH 100 mg daily		None demonstr before R 660 320
4 59 yrs Purpura	Cortisone 100 mg daily	0 5 before R 6 0 1 4	

Each figure represents the value of an individual determination made at 6–12 day intervals

treated with ACTH and in one treated with cortisone We selected male patients in order to avoid trouble with endogenous ovarian secretions

In case 1 and 2 high pregnanediol excretion determined by the method of Marrion et al, was found during therapy with ACTH This would suggest that ACTH stimulated secretion of 11 desoxy

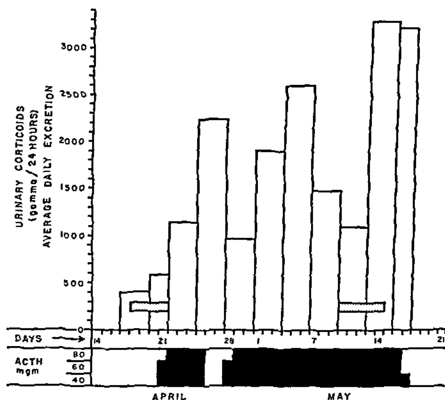


FIG 5 Excretion of formaldehydogenic steroids during treatment with ACTH hepatic cirrhosis
Crosshatched bars indicate menses

corticosterone or progesterone in appreciable amounts However in case 4 pregnanediol was found increased during treatment with cortisone in this case the pretreatment excretion level was determined and found to be almost zero

In case 2 and 4 high estrogen excretion determined by bioassay was found during administration of ACTH in case 3 none was demonstrable (minimum level tested for 30 International Units) before treatment

Another observation concerns the influence of menstruation on excretion of formaldehydogenic steroids (fg steroids) during ACTH

therapy The first case was one of severe hepatic cirrhosis studied jointly with Dr W P Havens Jr In this girl the urinary excretion of formaldehydogenic steroids rose during therapy with ACTH (80 mg daily) At the time of her menses the excretion decreased and rose promptly thereafter (Fig 5) This observation was made accidentally in a study of effects of ACTH in hepatic disease We wondered whether the impaired liver function had anything to do with the menstrual decline of formaldehydogenic steroid excretion

We therefore made a similar study on a girl treated with ACTH for rheumatoid arthritis (Fig 6) Again at the time of menstruation the formaldehydogenic steroid excretion took a nose dive

EXCRETION OF FORMALDEHYDOGENIC STEROIDS DURING ACTH THERAPY IN RHEUMATOID ARTHRITIS INFLUENCE OF MENSES

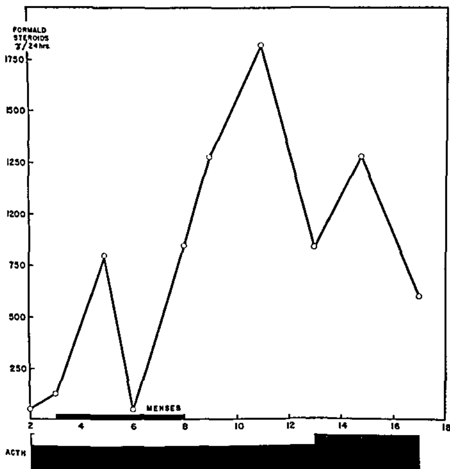


Fig 6

I wonder whether the data Dr Engel presented could be correlated to such an event Whether the decrease of formaldehydogenic steroid excretion represents an unspecific response to stress (unquestionably menstruation is a stress) or whether it is more specifically related to menstruation we do not know

DR GREGORY PINCUS I would like to ask Dr Engel if he has comparable data for patients receiving corticosteroids and whether they show any differences or similarities

DR LEWIS L ENGEL We have no comparable data on corticosterone I am sorry to say

8

Adrenal Function and Steroid Excretion in Disease*

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SLOAN KETTERING INSTITUTE FOR CANCER RESEARCH AND MEMORIAL CENTER FOR CANCER AND ALLIED DISEASES AND CORNELL UNIVERSITY MEDICAL COLLEGE NEW YORK

The adrenal hormones whether administered or secreted by the gland under stimulation provoke a dramatic response in many syndromes of disease but the mechanism of response is unknown The hormone may substitute for the decreased or faulty function of the adrenal gland but the fact that there is a response strongly suggests that in these diseases the needs of all tissues or of certain special tissues for adrenal hormone is not met by the normally secreted hormones in the cases in question

This situation can arise from two sources—(A) there is either an absolute or a relative deficiency in the production of steroid hormones by the adrenal gland or (B) there is a disorder in metabolism of the secreted steroids so that while sufficient hormone may be secreted by the adrenal it is altered before it reaches the essential tissues and thus a sufficient amount of the proper hormone is not available for the biochemical processes for which it is required

We have studied the human urinary steroid excretion patterns since these afford one means of studying hormone production by the adrenal gland We have compared the patterns of normal subjects with those found in disease and have sought to determine by the steroid excretion whether the adrenal responded like a normal or an abnormal gland when the stimulus of ACTH was applied

If we look at the steroid excretion patterns of normal subjects (Fig 1) there are four compounds which make up the major part of the ketonic steroids 11 keto etiocholanolone and 11 hydroxy androsterone which are derived from the 11 oxygenated adrenal hormones androsterone and etiocholanolone which arise from both gonadal

* This investigation was aided by grants from the American Cancer Society (on recommendation of the Committee on Growth of the National Research Council) the Commonwealth Fund the Anna Fuller Fund the Lillia Babbitt Hyde Foundation the Albert and Mary Lasker Foundation and the National Cancer Institute of the National Institutes of Health Public Health Service

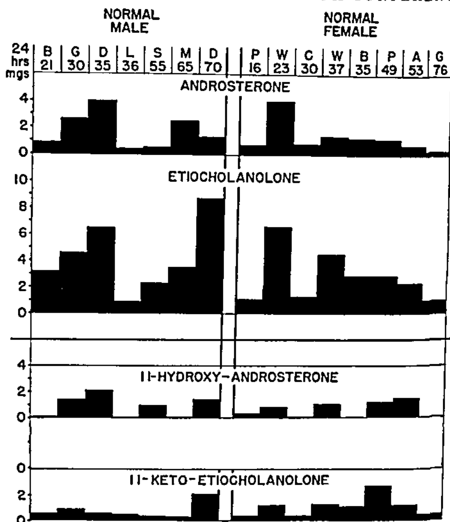


Fig 1

and adrenal hormones In addition to these major metabolites in normal man small amounts of other steroids are excreted such as pregnanolone androstanedione etiocholanedione as well as Compound F traces of E and some of their transformation products such as tetrahydro F and E The amount of each compound varies somewhat in different subjects and one may conclude that there are quantitative differences between individuals just as there are differences in height or weight or temperament but in the normal subject there are no qualitative deviations from the normal pattern

The steroid excretion pattern is quite abnormal in neoplastic disease (Fig 2) Not only do some normal compounds disappear completely but certain abnormal compounds of adrenal origin are found

in a significant number of cancer patients The conclusion to be drawn from these results is that there is an abnormal adrenal function or an altered metabolism of adrenal hormones when cancer is present

Can we draw any conclusion about adrenal function of normal or diseased subjects when their glands have been stimulated by ACTH? We are fortunate that through the cooperation of Dr Nathan W Shock of Baltimore we were able to compare the response of four

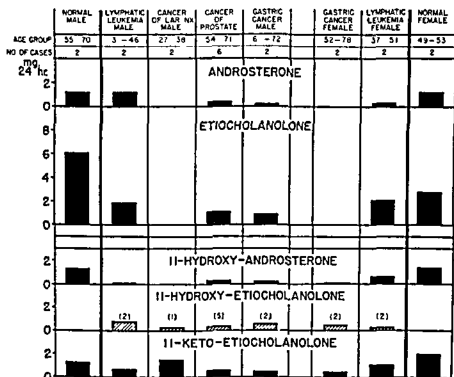


FIG 2

normal subjects in a metabolic experiment with the results we reported with leukemia patients last year at this conference Dr Shock s group conducted the metabolic studies while we studied the urinary steroids measuring both ketosteroids and formaldehydogenic steroids (Table I) The control period was 15 days after which the subjects received 100 mg ACTH per day in 4 divided doses for 12 days

It is remarkable that in three subjects the increases in ketosteroids and formaldehydogenic steroids increments are nearly identical and as Dr Shock reports the N and P excretion was similarly uniform The subject who showed no ketosteroid excretion did not show any changes in his N and P values indicating an unresponsive adrenal

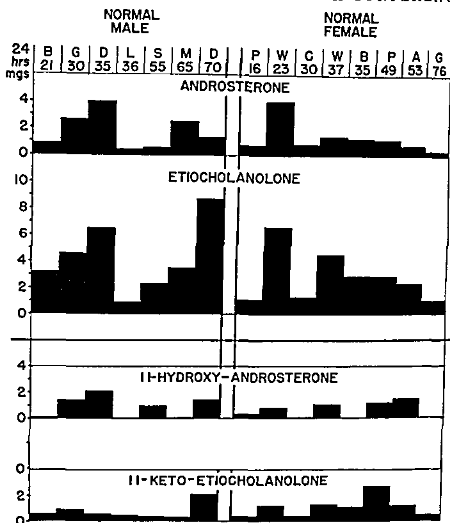


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the normal subjects. There was, however, a sharp difference in the level of formaldehydogenic steroids of the normal subjects and the patients with neoplasia. The control values of the cancer patients were over 10 times as high as those found in the normals and rose to much higher values after ACTH stimulation with much more variation in each individual. The results are summarized in Table III. The ketosteroid response is the same in the normal subject and in neoplastic disease, but there is a striking difference in the formaldehydogenic steroids. The average increase of formaldehydogenic steroids in the normal was 35 fold and in the neoplastic patients was seven fold.

Table III

	Ketosteroids (mg/24hr)		*Formaldehydogenic steroids (mg/24hr)*	
	Control	*ACTH ST*	*Control*	*ACTH ST*
		Average		
NORMAL	20.3	50.7	0.3	10.4
NEOPLASTIC DISEASE	16.5	50.9	3.8	26.6
		Ratio		
NORMAL	1.0	2.5	1.0	34.7
NEOPLASTIC DISEASE	1.0	3.1	1.0	7.0

It seems reasonable to assume that the adrenals in the neoplastic patients were already in a state of alert. Yet these same adrenals under stimulation were able to exhibit a considerable increase in production of hormones which yield formaldehydogenic steroids in the urine. The values reached were over twice the level of the normals.

A study of the individual steroids excreted before and during the stimulation of adrenal function by ACTH should give more precise information of hormone production and metabolism. The comparison of the results with those obtained after the administration of cortisone and other adrenal hormones should shed further light on the mechanism of steroid hormone action. The results obtained after adrenal stimulation by ACTH are shown in Fig. 3. The pattern of 6 urinary steroids before, during and after ACTH administration in three patients with neoplastic disease is compared with normal persons of the same age group. There was an increased excretion of both

Table I

NORMALS

ketosteroids (mg./24hr.)		formaldehydogenic steroids (mg./24hr.)	
Control	12 Days ACTH	Control	12 Days ACTH
22.7	58.1	0.3	8.1
20.2	60.7	0.4	12.4
23.1	29.8	0.2	7.1
15.0	54.0	0.3	14.1
Average			
20.3	50.7	0.3	10.4
Ratio			
1.0	2.5	1.0	34.7

Similar unresponsiveness in several patients to ACTH administration has been reported by us during the past year.

A similar study of patients with neoplastic disease is shown in Table II. The ketosteroids both during the control period and during administration of ACTH were essentially identical with those of

Table II

NEOPLASTIC DISEASE

ketosteroids (mg./24hr.)		formaldehydogenic steroids (mg./24hr.)	
Control	12 Days ACTH	Control	12 Days ACTH
14.7	63.6	4.2	11.9
16.6	42.7	1.7	37.8
17.0	41.7	5.4	24.0
24.7	32.6	1.0	18.3
16.5	38.6	2.4	25.5
10.7	48.6	—	—
16.0	60.8	10.5	40.4
15.1	55.0	0.2	11.9
17.1	74.9	5.0	43.0
Average			
16.5	50.9	3.8	26.6
Ratio			
1.0	3.1	1.0	7.0

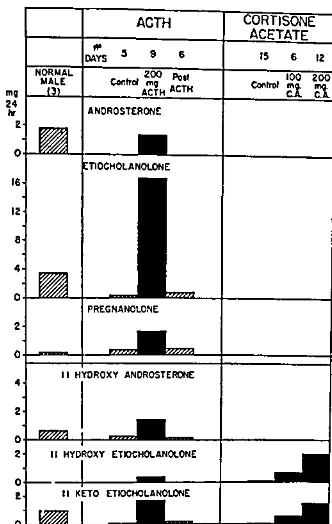


FIG 4

compound F The isolation of increased amounts of 11-oxygenated steroids is experimental proof that the side chain of cortisone is oxidized and steroids with 19 carbon atoms are produced In addition to these urinary C_{19} compounds *with* 11 oxygen function we find after cortisone as well as after ACTH administration increased amounts of compound F and E and two reduction products of both hormones These were tetrahydro E and tetrahydro F (Fig 5)

The isolation of these two compounds not only establishes their relation with the precursor hormones but illustrates that the *first step* in the degradation is a reduction of the conjugated ketone group in ring A This is of great physiological importance since these reduc

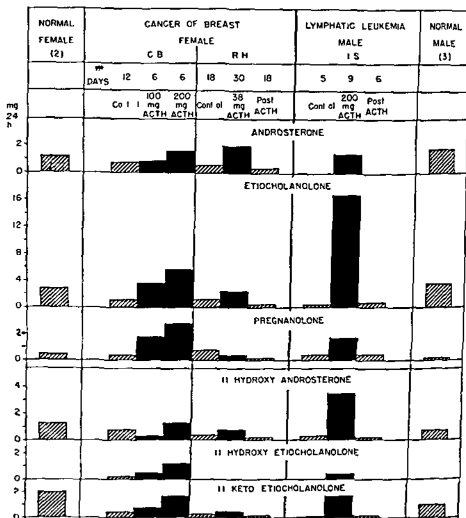


FIG 3

11 oxygenated and 11 desoxy compounds during adrenal stimulation

A comparison of the excretion pattern of 6 typical ketosteroids after ACTH stimulation with a pattern after cortisone administration is shown in Fig 4 The two patients had lymphatic leukemia The patient who received 100 mg of cortisone for 6 days and 200 mg for 12 days excreted in his control period only traces of amounts of the 6 compounds After administration of cortisone a striking increase of the 11 oxygenated urinary metabolites was observed There was *no* increase of the 11 desoxy compounds during cortisone treatment in contrast to the great increase after ACTH

From the above we can conclude that the adrenals produce other hormones or steroid precursors than cortisone or the closely related

is metabolized to these two end products A more likely explanation is that compound S (11 desoxycortisone) or 17 hydroxy progesterone (11 21-desoxycortisone) can be metabolically oxidized to androsterone and etiocholanolone Indeed using isotopically labelled C_{21} steroid hormones of the 11 desoxy type in a cooperative study with Dr Gallagher and Dr Fukushima the transformation to C_{19} 17 ketosteroids has been observed This is proof that the 11 desoxy hormones of the human adrenal contribute to the urinary androsterone and etiocholanolone Since both these compounds are increased by ACTH stimulation it is almost certain that their C_{21} 11-desoxy precursors are normal secretions of the adrenal gland The metabolism of these compounds then is very similar to the metabolism of corti

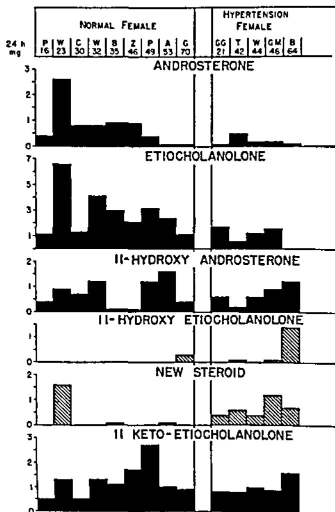


FIG 6

tion products are much less active biologically than the hormones themselves The have no therapeutic activity when given in the same amounts as the hormones

A third metabolite related to cortisone and compound F was isolated from urine This substance was 21 desoxy tetrahydrocortisone (3α 17α dihydroxypregnane 11 20 dione) (Fig 8) and was obtained both after cortisone as well as ACTH administration The reduction of the side chain seems to occur after the reduction of the A ring

In summary cortisone in its metabolism undergoes a series of reductions to more saturated compounds with the same number of

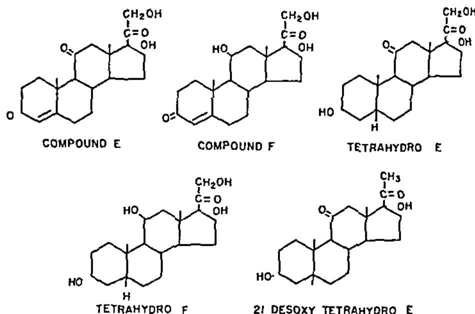


FIG 5

carbon atoms In addition however there is also oxidative loss of the side chain with the formation of the C_{19} steroids still having the 11 oxygen function as has been shown previously There is no evidence that during metabolism a loss of the 11 oxygen function occurs We are forced to assume therefore that the precursors of the 11 desoxy steroids—androsterone etiocholanolone and pregnanolone—are adrenal hormones without 11 oxygen function *Pregnanolone* may derive from progesterone and DOC both of which have been isolated from adrenal glands We have no evidence that the side chain is lost with the formation of C_{19} compounds

The precursors of the increased amounts of androsterone and etiocholanolone are still not accounted for There is no doubt that their precursors after ACTH stimulation are adrenal steroids It is unlikely that the adrenals produce testosterone a compound which

urine but the amount of each compound is small actually at the lowest level found in normal subjects The most significant finding however is the presence of a compound which from its characteristic structure is an adrenal hormone metabolite—17 hydroxy pregnanolone This compound has not been found in the urine of any of the twenty-eight normal subjects studied It has been found quite regularly in cases of adrenal hyperplasia and adrenal tumors Therefore this patient with arthritis had an adrenal dysfunction

We are aware that from a single instance one cannot draw the conclusion that in *all* cases of arthritis there is adrenal dysfunction There is *no doubt* however that adrenal dysfunction was present in this patient The patient made a dramatic temporary recovery under treatment with cortisone Studies of his urinary steroid pattern as affected by this treatment with cortisone are under way as are studies of the excretion pattern of the same patient under ACTH therapy all of the results of which may reveal some interesting leads

SUMMARY

We have studied the adrenal function before and after stimulation with ACTH and discussed the pathway of metabolism of adrenal hormones We have given evidence that the adrenal hormone excretion is changed in neoplastic disease hypertension and in one case of arthritis

We conclude that dysfunction of the adrenal is an important factor in these syndromes and that further study will greatly expand our knowledge of the specific role of the hormones and of the adrenals in health and in disease

The authors express their deep appreciation to Dr Thomas F Gallagher for his generous and valuable assistance in the preparation of this manuscript

DISCUSSION

DR WILLIAM Q WOLFSON Figure 8 summarizes observations made last year by Dr Robinson and Dr Duff during a study in which the effects of cortisone were compared with those of aqueous adrenal cortical extract An equal number of glycogen units was given daily in both periods Clinical and metabolic effects were quite comparable with one exception The aqueous adrenal cortical extract produced a marked increase in 17 ketosteroid excretion while the cortisone did not (Fig 8)

It is entirely reasonable to assume from this result that adrenal

sone i e they are in part degraded to C_{19} steroids and in part excreted as reduced C_{21} steroids

Let us now turn to other syndromes than cancer to inquire whether an abnormal production or metabolism of steroid hormones is present Such a syndrome is essential hypertension and it can be seen in Fig 6 that two abnormal steroids are excreted in a significant number of patients

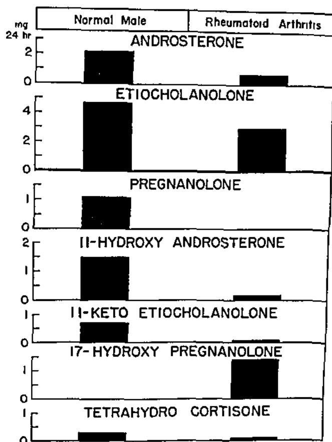


Fig 7

Arthritis however is one outstanding condition which responds strikingly to adrenal hormone administration as well as to adrenal stimulation by ACTH Exhaustive disputation has not decided the status of adrenal function of the arthritic patient

Through Drs Sprague and Mason of the Mayo Clinic we have obtained the urine of a patient with arthritis and in Fig 7 the steroid excretion pattern of the arthritic is shown in comparison with that of a normal subject With the exception of pregnanolone all the com pounds excreted by the normal male are also present in the patient s

9

Adrenal Function During Pregnancy and the Effect of ACTH During Pregnancy

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NORMAL PREGNANCY

The report by Venning (1946) has stimulated interest in the role of the adrenals during pregnancy Tobian (1949) and Jailer (1950) using different techniques have confirmed the observation that there is a marked increase in urinary corticoids during pregnancy Our studies demonstrating a rise in corticoids and a much smaller increase in urinary excretion of 17 ketosteroids are presented in Fig 1 Davis has also shown a decrease in circulating eosinophils during the course of pregnancy only to rise after delivery

PREGNANCY IN ADDISON S DISEASE

Our interest in this field was originally aroused by the opportunity to study several Addisonian patients during pregnancy This experience has been presented by Knowlton Mudge and Jailer (1949) Subsequent to this one of the patients has undergone another pregnancy and she was re investigated with newer methods for studying adrenal function She was admitted at approximately monthly intervals to the metabolism ward for study Pellets of desoxycorticosterone had been implanted six months previous to the onset of this pregnancy and when it was felt that they had been absorbed she was maintained on 2 mg DcA daily This was supplemented with varying amounts of added sodium chloride During the last trimester of pregnancy it was necessary to decrease the amount of added salt

During both pregnancies there was a marked increase in 17 ketosteroid excretion At the time of the first pregnancy 17 ketosteroids were determined according to the method of Pincus with antimony trichloride and during the second the colorimetric method employed was the Holtorff Koch modification of the Zimmerman Reaction

cortical extract contains the 17 ketosteroid precursor which is responsible for the marked increase in 17 ketosteroid excretion seen with ACTH This precursor is not substances A B E F S or desoxycorticosterone for none produce a comparable increase in urinary 17 ketosteroids The 17 ketosteroid precursor contained in adrenal cortical extract would appear to be relatively inert metabolically since no differential metabolic changes attributable to its effects were observed in this study

Paper chromatographic studies of the 17 ketosteroids excreted after administration of ACTH have shown an interesting difference in the qualitative time relations of one of the fractions With suitably

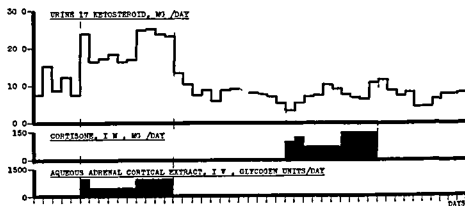


FIG 8

large doses an increase in the usual monoketonic monoalcoholic steroids may be seen clearly on the chromatogram

Following withdrawal of ACTH the concentration of this monoketonic fraction falls to below control values and then returns to its baseline Very different behaviour is observed in the case of one of the diketonic 17 ketosteroids This steroid increases during the administration of ACTH and when ACTH is withdrawn its concentration continues to increase for some days Maximum output is observed at a time when all metabolic indices indicate a relative lack of 11 17 oxysteroids to be present Clearly therefore this substance cannot be a derivative of a steroid with 11 17 oxysteroid activity but must arise from an inert substance or from one which has activities opposite to those of the 11 17 oxysteroids Its behaviour is in fact entirely consistent with that expected of an intermediate in adrenal cortical steroid metabolism The position of this steroid on the chromatogram is consistent with androstenedione or etiocholenedione The former is currently widely regarded as a possible precursor of at least three groups of adrenal steroids

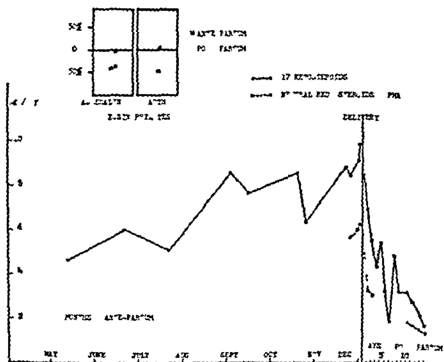


Fig 2

have focussed our attention on this organ The extraction procedure is difficult technically and probably quite inefficient To date we have a ketonic fraction of pooled premature placentae which gives the Zimmerman reaction reduces phosphorolybdic acid and more specifically liberates formaldehyde on incubation with periodic acid However it is probably not cortisone since in the dosages employed there was no deposition of liver glycogen in adrenalectomized mice In partition studies between benzene and water it appears to be more soluble in benzene It has not been crystallized as yet

Evidence of a protein like hormone similar to ACTH has been found in placental tissue These extracts are capable of reducing ascorbic acid in newborn rats according to a method previously described (Jailer 1950) and (in collaboration with Drs P E Smith and F Agate) it will reduce adrenal ascorbic acid in the hypophysectomized rat as well Its role in normal pregnancy is unknown

TOXEMIA OF PREGNANCY

It would thus appear that during pregnancy there is ample evidence of a temporary state of hyperadrenalism in women There is experimental evidence for a similar state in pregnant animals as well

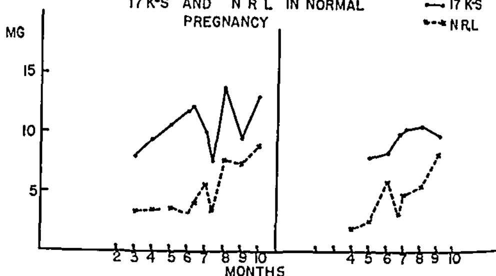


FIG 1 Daily excretion of urinary steroids in mgms determined at various times during course of pregnancy (N R L neutral reducing lipids or steroids)

Fig 2 demonstrates the increase noted in one patient Within 7-10 days postpartum these values fell to typical Addisonian levels The excretion of neutral reducing lipids was high in the last trimester and fell accordingly after delivery

The administration of 0 3 mg epinephrine on two separate occasions and 25 mg ACTH on one occasion resulted in at least a 40% decline in circulating eosinophils When these tests were repeated after delivery however there was no decrease in circulating eosinophils (Fig 2)

An attempt was made to rule out the fetal adrenals as a source of these steroids Urine was obtained from the male infant for the first three days of life An average of 0 6 mg 17 ketosteroid and 0 3 mg neutral reducing lipids per day were found These values were obtained during the time when the maternal values were still above her usual non pregnant values This seemed to us at least to rule out the fetus as the source

It was also possible that this patient had adrenal remnants which were being stimulated during pregnancy in some fashion Consequently 100 mg /day of ACTH were administered beginning the 11th postpartum day and no fall in eosinophils and no increase in 17 ketosteroids could be observed (Figure 3)

The only remaining source of these steroids would appear to be the placenta Previously chorionic gonadotropin estrogens and progesterone have been isolated from placental tissue Therefore we

After suitable control periods 70 mg ACTH (in 4 divided doses) were administered daily One patient received 150 mg cortisone daily

Several patients went into labor after but a few doses of ACTH These women are not included in this report Indeed we soon felt

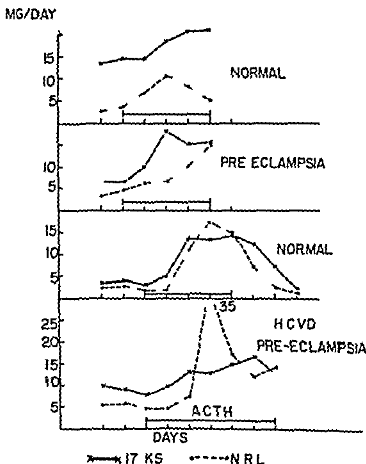


Fig 4

that ACTH had precipitated labor but more careful study showed this to be fortuitous and not causal Six patients were followed a sufficient length of time to attain all the physiological effects

There is conclusive evidence that the ACTH was physiologically active The eosinophils declined markedly often disappearing completely The 17 ketosteroid excretion values more than doubled The neutral reducing lipids reached exceedingly high values—40 mg/day in one patient (Fig 4) There was a retention of sodium al

The question arises whether there is any further alteration in adrenal cortical function in toxemia of pregnancy This pathological state is characterized by hypertension albuminuria and edema In recent literature there have been several speculative papers by Garrett Parviainen Soiva and Ehrnrooth claiming that the etiology of pre eclampsia and eclampsia is bound up with excessive secretion of adrenal cortical hormones Tobian has shown that pregnant women with excessive edema excrete about 46% more formaldehydogenic steroids than pregnant women with minimal or no edema There was no

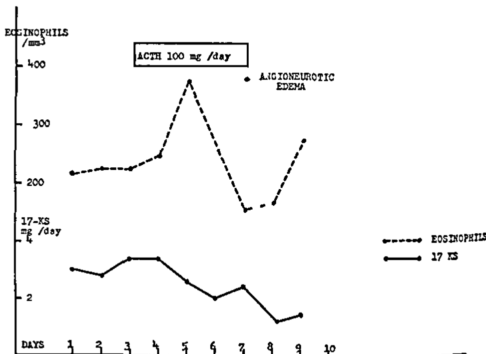


Fig 3

correlation however with the level of the blood pressure Consequently it seemed to be of interest to see what effects the administration of ACTH and/or cortisone would have on normal and pathological pregnancy

Patients in the last trimester of pregnancy were studied in the hospital and placed on the hospital salt poor diet The normal pregnant women were given 6 grams of sodium chloride in addition the pre eclamptic no added salt The latter patients on admission were placed on bed rest sedation salt restriction and magnesium sulfate intravenously when indicated This usually resulted in a marked clinical improvement with a decrease in edema hypertension and albuminuria After stabilization the study was instituted

both in early and late pregnancy have consistently responded with a much greater rise in fasting blood sugar The average rise was approximately 30 mg per cent and the smallest 15 mg per cent Five such results are shown in Fig 6 The lowest curve shows the minimal effect obtained The first curve indicates the changes in a diabetic pregnant woman

It should be emphasized that there is evidence for some impair

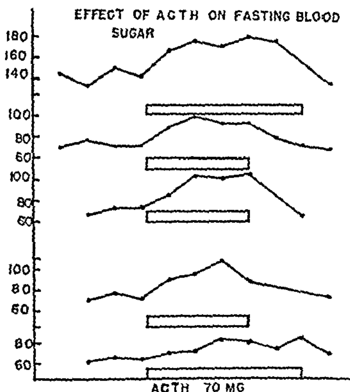


Fig 6

ment of carbohydrate metabolism during pregnancy A small percentage of women show a lowered renal threshold for glucose and others may even show impaired glucose tolerance

In summary there is evidence of increased production of adrenal steroids during pregnancy in both normal patients and Addisonians The possibility exists that at least part of these are of placental origin

The administration of ACTH and cortisone under the conditions of our experiment does not cause any alteration in the usual course of normal or toxemic pregnancy Studies are underway to ascertain more about the role of sodium in the etiology of toxemia

though in the pre eclampsia patients the control urine sodium values were low due of course to salt restriction

However under the conditions of this experiment ACTH and cortisone had no effect upon the clinical course of normal or toxemic pregnancy There was no increase in albuminuria no edema formation no effect on serum uric acid no effect on resting blood pressure

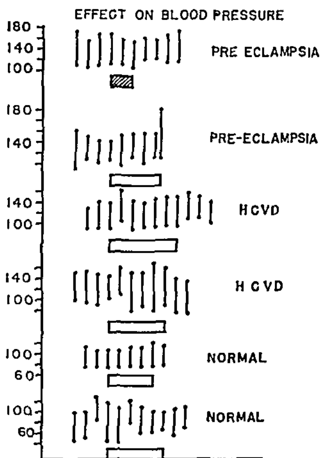


Fig 5 Horizontal bars represent period of ACTH administration Vertical lines represent average daily resting blood pressure

sure and but minimal weight increase (Figure 5) One toxemic patient after stabilization on ACTH showed a rise in blood pressure during labor however this is known to occur normally

The only finding worthy of note was a consistent rise in fasting blood sugar in all patients studied Our previous experience with unselected individuals given adequate doses of ACTH has been an occasional rise in fasting blood sugar Pregnant women however

incidental to the study of physiological variations in tail blood eosinophil levels

A highly significant and regular daily variation in eosinophils was recently reported in five different stocks of mice studied with Dr Visscher in the Department of Physiology of the University of Minnesota Fig 8 presents in part unpublished data extending the finding to the B_1 subline of the C , Black stock In view of the diurnal rhythm one should consider the effects of pregnancy at the time of the midnight low as well as during the morning high It can

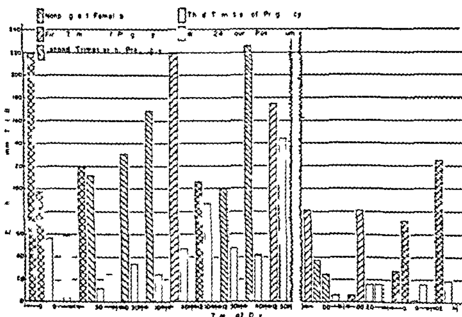


Fig 7

be seen from the first slide that a marked eosinopenia in the third trimester of pregnancy can be detected at both these periods of the day in the Z_b stock of mice

In conclusion I call attention to the extent of the diurnal rhythm which in some stocks of mice and under the controlled conditions reported elsewhere amounted to a regular difference of about 80% between the levels determined from 6 to 10 A M and those obtained in the period from 9 P M to 1 A M Such diurnal rhythms impress us as a more general phenomenon They have been reported for the excretion of 17 ketosteroids and of neutral reducing lipids in the urine of men and for the lipid content of the adrenal cortex in mice Exact indication of the period of the day at which studies

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DISCUSSION

DR KARL E PASCHKIS At the present moment we have a patient quite similar to that of Dr Jailer s under study a woman with Addison s disease who was studied before she was pregnant then she was studied again during pregnancy

The studies so far show that her formaldehydogenic steroids before pregnancy were 200 micrograms per 24 hours and toward the end of the third month of pregnancy they are up close to 800 which is about the top level of normal for formaldehydogenic steroids

DR FRANZ HALBERG (University Hospital and University of Minnesota Medical School Minneapolis) I should like to present data on eosinophil levels during pregnancy as collected from the Z_1 stock of mice* and shall also use the occasion to emphasize the importance of a physiological phenomenon of possible interest to this audience

During the third week of pregnancy we observed a marked eosinopenia in contrast to the levels determined under standardized conditions and at comparable times both in non pregnant females of the same stock and of similar age (± 20 days) and in pregnant animals during the first and second week of pregnancy Tail blood from controls was collected for an eosinophil count within five minutes from the blood letting of mice in their third trimester of pregnancy

Typical results are seen in Fig 7 The light bars represent levels obtained in mice during the third week of pregnancy The cross hatched bars represent counts from animals in the second or first trimester of pregnancy and some non pregnant females As you can see the time scale of the bar diagram is not constant The figures were obtained at specific times of the day and represent a finding

* Fostered line of C_3H obtained through the courtesy of Dr J J Bittner

10

The 48 Hour ACTH Test in Pregnancy and Cortisone Response in Toxemia*

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BOSTON LYING-IN HOSPITAL, FLORENCE CRITTENTON HOME AND HARVARD MEDICAL SCHOOL, BOSTON

Pregnancy is a temporary 40 week state of increased pituitary and adrenal activity Associated with the increase in function there is in some cases hypertrophy and hyperplasia of each of these glands In addition an increase in basal metabolic rate protein bound iodine and cholesterol has been observed in some cases during pregnancy The marked change in the glucose tolerance test as pregnancy progresses has been described Although many of these metabolic changes in pregnancy must be related to changes in adrenal cortical function data to support the relationship are meager Venning described an increase in urinary corticoids and 20 ketosteroids as pregnancy progressed No increase in 17 ketosteroids was detected Rath and others have noted the progressive decrease in circulating eosinophils during pregnancy

This report is divided into two parts The first describes the effort to evaluate pituitary and adrenal function by 48 hour ACTH stimulation during pregnancy The second part describes the results of cortisone suppression of adrenal and pituitary activity in pregnancy complicated by hypertension and albuminuria

48 HOUR ACTH TEST IN PREGNANCY

Two clinically normal primiparae cooperated in the studies No attempt was made to change their dietary regimen or to restrict their activities in any manner The same kind and quantity of food was eaten by each of these women at each meal during the entire period of study The patients were ambulatory in the Florence Crittenton Home and during the 48 hour test periods their housework duties were not interrupted

* Aided in part by grants from the Boston Lying in Hospital Associates the Milton Fund Harvard University and Charles H Hood Foundation Boston

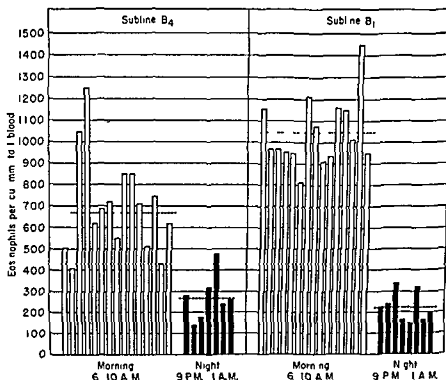


Fig 8

directed towards the elucidation of problems related to homeostasis are undertaken is therefore desirable It appears as a sine qua non for the determination of eosinophil levels in the intact laboratory mouse where within each 24 hours this rhythm may bring about a change from over one thousand cells to 200 cells per cu mm tail blood

a progressive increase in the excretion of 17 ketosteroids does occur during the sixteen week period of study

Figure 2 illustrates the results in the second patient studied This patient was *clinically normal and except for excessive weight gain with edema she did not differ from the previous patient*

A totally different type eosinophil response is noted During the first twelve to eighteen hours of ACTH stimulation a decrease in circulating eosinophils occurs but during the remaining thirty to thirty six hours of the study period there is a progressive increase in

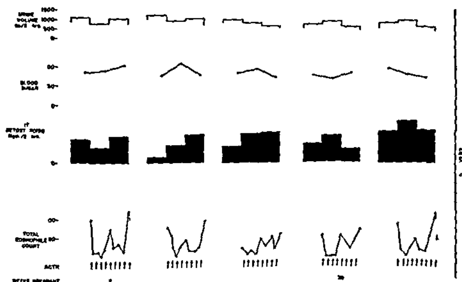


Fig 2 Response to ACTH stimulation during pregnancy complicated by excessive weight gain

the cell count In each instance it is noted that the fasting counts at the end of the 48 hour period of stimulation is greater than the pre stimulation counts The counts were all taken under the same basal conditions

This response rebound or eosinophil escape was noted in all tests in this case If at the end of the 48 hour period however an injection of 50 mg is given decrease in the eosinophil count occurs This is illustrated by the broken lines in the figure Except for the first test period no increase in the 24 and 48 hour fasting blood sugar is detected The 17 ketosteroid excretion except for the second study period is not increased during the five periods of stimulation No sudden weight gains are observed during the tests However this

Beginning at the twenty third week the 48 hour ACTH test was performed at three to four intervals throughout the remainder of the pregnancy An attempt was made to have the last prepartum observations as close to the onset of labor as possible Five tests were completed on each patient during the last sixteen weeks of pregnancy

Total eosinophil counts were done as described by Dunger while 17 ketosteroids were estimated according to the method of Callow Blood sugar was determined by the Folin Molinos method

The same lot of ACTH was used in the entire study In the first test on each patient an initial injection of 25 mg of ACTH was

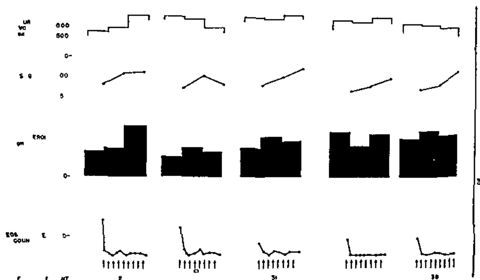


FIG 1 Response to ACTH stimulation during normal pregnancy

given followed by 10 mg every six hours for 48 hours During the last four tests 25 mg was injected every six hours for 48 hours making a total of 200 mg given during the test period

RESULTS

Figures 1 and 2 illustrate the response obtained when the adrenal cortex is stimulated by ACTH at regular intervals during pregnancy The first patient studied (Fig 1) shows a prompt and sustained decrease in circulating eosinophils during the injection of ACTH This low count is maintained during each of the 48 hour periods of observations There is a moderate increase in the fasting blood sugar in all test periods except the second The 17 ketosteroid excretion during ACTH stimulation is increased in the first period only However

signs and symptoms of the disease The three cases described below are not reported primarily as a method of treatment but are attempts to learn more about the physiological changes in pregnancy complicated by toxemia It is our hope that a better understanding of pituitary and adrenal function during toxemia will result in improved treatment of the disease The edema hypertension albuminuria and water retention of this complication of pregnancy suggests that increased pituitary and adrenal activity might be related to their production Cortisone was therefore used in an attempt to suppress

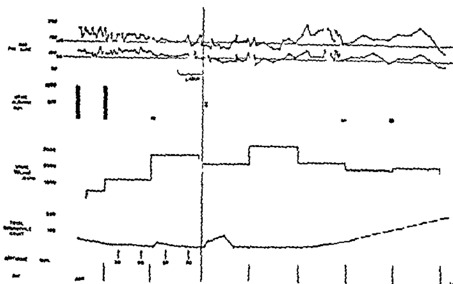


Fig 4 Cortisone response of severe pre eclampsia

these activities and to determine if the signs and symptoms of pregnancy toxemia might be changed

Figure 4 illustrates the results in the thirty year old gravida 3 para 2 who was admitted with severe pre eclampsia when thirty weeks pregnant Her first pregnancy was complicated by toxemia and a second pregnancy was uneventful Three members of her family have severe hypertension A maternal aunt had toxemia with convulsions during one pregnancy

During the twenty ninth week of the present pregnancy a sudden onset of severe headaches marked visual disturbances hypertension and generalized edema occurred Because of vaginal bleeding in the first trimester the patient had been placed on diethylstilbesterol all medication was discontinued and 50 mg *cortisone* at 12 hour intervals now started After twenty hours the patient had an unusual sense of well being Symptomatic improvement was marked A pro

patient s total weight increase during the 16 weeks of study was excessive by normal standards

A third patient Figure 3 exhibited a similar lack of response to stimulation and three weeks following the test developed moderately severe pre eclampsia When the signs and symptoms of this complication of pregnancy appeared very few eosinophils were detected in the peripheral blood The absence of a normal response to exogenous ACTH stimulation suggests high endogenous pituitary hormonal

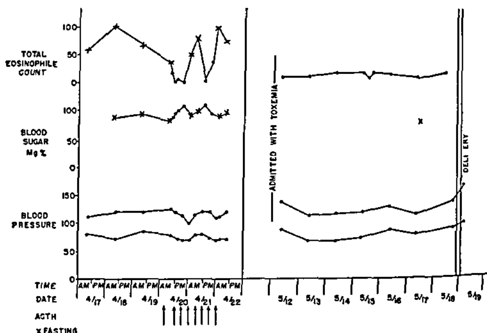


Fig 3 Forty eight hour ACTH test during pregnancy complicated by excessive weight gain and edema Three weeks after the completion of the test the patient developed pre eclampsia

production and the low eosinophil counts seemed indicative of increased adrenal cortical function

CORTISONE RESPONSE IN TOXEMIA

The second part of this report describes the use of cortisone in three patients with toxemia of pregnancy The evaluation of treatment in toxemia is difficult The signs and symptoms of the disease are variable and remissions occur spontaneously Hospitalization followed by minimal sedation may result in marked improvement even in severe pre eclampsia The onset of labor occasionally alleviates the

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DISCUSSION

DR R R MARGULIS A limited number of patients were studied at the Henry Ford Hospital In one group were women with normal pregnancies who were given ACTH near term to see if it were possible to induce labor One woman with a retained dead fetus was treated with the same objective

In the second group ACTH and cortisone were administered to determine their possible value in therapy of mild and severe eclampsia

We were not able to prove conclusively that ACTH would induce labor

The eclamptics felt better on cortisone treatment as Dr Caton mentioned We did not see any striking change for better or for worse in the blood pressure There was no change in total daily urinary albumin output Single urine specimens frequently showed a decrease from 1 + to 1 or 2 + but this was due to the increased volume of the urinary output

We had one patient admitted to the Emergency Room in convulsions Under mild sedation and cortisone therapy the convulsions stopped and there was a striking subjective improvement Five days later she spontaneously went into labor Again there were no demonstrable changes in either the albuminuria or hypertension

DR JOSEPH W JAILER The only thing I would like to say is that we too thought that ACTH was putting women into labor but when we really tested it we found it didn t do so at all

fuse urine output was almost free of albumin Spontaneous labor occurred after thirty hours of cortisone treatment with delivery of an active 2–12 infant Both mother and baby were discharged well

Patient #2 developed eclampsia during the twenty second week of pregnancy Her first convulsion occurred one hour after admission to the hospital and thirty minutes after the initial injection of cortisone Magnesium sulfate had been given in addition to cortisone This combination of therapy stabilized the patient and no further convulsions occurred No change in urine output or albuminuria was noted After a short period of stabilization the pregnancy was surgically terminated

Patient #3 was an obese multipara whose pregnancy was complicated by severe hypertension and superimposed toxemia At term with ruptured membranes and no labor she was admitted semi stuperous *completely amnesic and disoriented Twelve hours after* starting cortisone the patient was rational and oriented Cortisone was continued in 50 mg doses at 12 hour intervals A moderate fall in blood pressure was noted In spite of restriction of fluids during this twelve hour period to 250 cc of hypertonic glucose a 1300 cubic centimeter diuresis occurred All visual disturbances had disappeared and she had no complaints Cortisone was discontinued after 48 hours Labor and uneventful delivery of a normal infant occurred two days later

SUMMARY

The results in two of the three patients are encouraging but several limitations of such hormone suppression are noted First if it is to be adequate a certain duration of time is necessary in order to accomplish the suppression The lack of response in the patient with eclampsia illustrates this factor Second the immediate effects of cortisone may possibly precipitate an exacerbation of the condition rather than result in improvement

Perhaps if in the less severe cases of pre eclampsia the pituitary and adrenal can be effectively suppressed the clinical course of the disease might be changed so that the pregnancy can be continued or terminated with safety

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PART II

KIDNEY AND ELECTROLYTES

11

Interactions Between the Adrenocorticotrophic Hormone (ACTH) and the Somatotrophic Hormone (STH) in Respect to Their Effects upon the Kidney and the Cardiovascular Apparatus*

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It is now a generally accepted fact that during exposure to stress large amounts of corticotrophic and corticoid hormones are discharged into the blood as an integral part of non-specific defense during the general adaptation syndrome [1,2,3] There is considerable dis agreement however concerning our hypothesis according to which during adaptation to stressor agents an intoxication with endogenous anterior pituitary hormones or corticoids could predispose the organ ism to the development of disease and thus result in diseases of adap tation

Animal experiments revealed that malignant nephrosclerosis periarteritis nodosa as well as morphologic changes reminiscent of the hypertensive and rheumatic diseases can be produced by min eralo-corticoid substances such as desoxycorticosterone (DCA)[4] or desoxocortisone (Reichstein s compound S)[5] Essentially similar cardiovascular and connective tissue lesions of the so called collagen disease type have also been reproduced in animals by a lyophilized anterior pituitary preparation (LAP)[6,7]

* This investigation was performed with the help of grants from the National Heart Institute (U S) the National Research Council of Canada and the Canadian Arthritis and Rheumatism Society

In order to facilitate the survey of the numerous animal experiments which had to be performed in order to arrive at this conclusion we shall first summarize the results of each experimental series and then attempt their correlation and evaluation in the section Discussion This procedure will also help to distinguish clearly between the established facts on the one hand and the hypothetical evaluation of their significance on the other

EXPERIMENTAL PROCEDURE

Although we make no attempt to outline our techniques in detail here it is pertinent to mention that generally speaking all these experiments were performed on female piebald rats weighing 100–140 gm The right kidney was invariably removed on the day preceding the administration of the first hormone injections The animals received a powdered purina fox chow diet and were given 1% NaCl solution instead of drinking water The unilateral nephrectomy and the NaCl supplement were administered because these factors had previously been shown to increase sensitivity to the mineralo-corticoid actions of LAP or DCA although they do not produce any toxic effects in themselves [1]

ACTH (Armour) was usually administered at a dose level corresponding to 6 mg per day of the Armour standard This amount was administered in six injections (4 hours apart) during the day and night

STH [purified preparations supplied by Dr C H Li (Berkeley) Armour (Chicago) Organon (Oss) Horner (Montreal)] was given at a dose level of approximately 5 mg per day divided into 3 injections

Cortisone (Merck) and DCA (Schering) were given in the form of micro-crystalline suspensions (25 mg per cc in saline with added suspending agents) 2 5–5 mg per day in a single dose

LAP was prepared from dissected cattle anterior lobe The resulting powder was suspended in saline (40 mg per cc) and injected at the dose level of 40 mg per day in two divided doses

All injections were administered by the subcutaneous route

The duration of these experiments was usually one month unless the hormone combinations proved to be so toxic that the animals died with overdosage manifestations before that time

PRINCIPAL FACTS OBSERVED

1 LAP is rich in STH

It will be recalled that LAP is merely a dried cattle anterior pituitary powder which contains all the adenohypophyseal princi

The resemblance between the actions of DCA and LAP became even more obvious when it was found that both these hormone preparations predispose the rat to the production of experimental arthritis [8] and to anaphylactoid reactions [9] As a working hypothesis we assumed therefore that LAP may act by virtue of its corticotrophic effect perhaps by stimulating the production of mineralo-corticoids through the adrenals

However later when adequate amounts of purified ACTH (Armour) became available to us it was noted that even the highest tolerable doses of this hormone failed to reproduce the above mentioned actions of LAP Thus ACTH causes no nephrosclerosis it exerts only irregular effects upon the blood pressure and far from eliciting collagen disease and predisposing to arthritis or allergy it actually inhibits their development both in experimental animals and in man Pure synthetic gluco-corticoids (e g cortisone) act essentially like ACTH not like DCA or LAP Apparently, ACTH is predominately a gluco corticotrophic hormone that is one which stimulates the adrenal to produce gluco-corticoids Hence obviously the active principle in LAP could not be identical with ACTH It remained to be shown whether the typical LAP effect is due to one of the other known anterior pituitary hormones to a hitherto unknown hormone or to a combination of several hypophyseal principles In the meantime the agent in question was merely designated as the LAP factor or X factor of the pituitary [9,10]

A large number of experiments performed in our Institute during the last few months have led us to conclude that this LAP factor is identical with the so-called growth hormone or somatotrophic hormone (STH) Limitations of time prevent us from reporting upon all these experiments in detail here today but in view of the important inter actions between STH and ACTH we felt that a brief synopsis of pertinent investigations would be of some interest to the participants of this conference Those interested will find complete descriptions of these experiments in the references cited *

EXPERIMENTAL OBSERVATIONS

The principal object of this communication is to review experimental observations which led us to conclude that STH is the anterior pituitary factor responsible for mineralo corticoid actions Hence in a sense it represents the natural counterpart of ACTH the hormone primarily responsible for gluco corticoid secretion

* Many of these publications are still in press at the time this manuscript is submitted hence the bibliographic data are of necessity incomplete However we have been assured that all these papers will appear in 1951 and since the journals are mentioned the reader will encounter no difficulty in locating them as soon as they will appear

4 LAP produces hyalinosis

All the above mentioned toxic actions of DCA can be reproduced with crude anterior pituitary extracts or LAP The main difference between the syndrome produced by DCA on the one hand and the pituitary preparations on the other is that the latter rarely elicit periarteritis nodosa in the free mesenteric arteries although they do cause such vascular lesions in the heart and sometimes in the pancreas Furthermore LAP elicits a syndrome of splanchnomegaly (marked enlargement of the spleen liver and kidney) this is much less pronounced after DCA administration The splanchnomegaly was attributed to the STH content of the LAP especially since it is regularly accompanied by a marked increase in somatic growth [6,7,8]

5 STH produces hyalinosis

Electrophoretically pure STH reproduces all the above mentioned actions of LAP From this it was concluded that STH is the hitherto unidentified LAP factor which imitates mineralo corticoid actions Like LAP STH does not tend to produce any mesenteric periarteritis nodosa and causes a pronounced hypertrophy of the adrenals in not hypophysectomized animals [10] However after ablation of the pituitary STH does not tend to cause any adrenal enlargement nor does it produce hyalinosis [11] Apparently endogenous ACTH participates in the production of hyalinosis by STH Perhaps the injection of STH elicits a discharge of ACTH from the animal s own pituitary just as FSH causes an LH discharge and vice versa

6 ACTH does not produce hyalinosis

Even toxic doses of ACTH have no tendency to elicit the hyalinosis syndrome It does cause marked hyperemia of the renal glomeruli which in extreme cases may lead to glomerular hemorrhages and the disintegration of some nephrons However these lesions are so different from those produced by DCA LAP or STH that we may safely exclude ACTH as the agent primarily responsible for the production of DCA like mineralo-corticoids [8,12]

7 Cortisone does not produce hyalinosis

Overdosage with cortisone elicits the same renal changes (essentially characterized by glomerular hyperemia) as ACTH This is in agreement with the generally accepted view that ACTH is primarily

ples as well as much accompanying non hormonal protein material However the preparation is particularly rich in STH as shown by the fact that it considerably promotes somatic growth as well as the proliferation of connective and lymphatic tissues All these actions are shared by electrophoretically pure STH [11 12] This consideration led us to suspect that the mineralo corticoid like actions of LAP may be due to its STH content

2 Adrenalectomy prevents the mineralo corticoid actions of LAP

Earlier observations had shown that in adrenalectomized rats maintained on cortical extracts LAP does not elicit its usual toxic effects upon the cardiovascular and renal tissues Thus it became extremely probable that the effect of our pituitary preparation is actually mediated through the adrenal cortex [7 13 14] However adrenal ectomized rats even when given fairly high amounts of adrenal cor tical extracts do not withstand LAP treatment very well presumably because the latter tends to produce subcutaneous abscesses and con tains much toxic protein material Because of the poor condition of the experimental animals the above findings were difficult to evalu ate In more recent experiments in which adrenalectomized rats were maintained in excellent shape with cortisone LAP still failed to exert mineralo corticoid actions In this series control rats received the same amount of LAP and cortisone as the adrenalectomized ani mals yet the former died with manifest nephrosclerosis and myocar ditis at a time when their adrenalectomized partners were still in per fect shape and showed no macroscopic or even microscopic evidence of cardiovascular or renal damage Here we were faced with the rather singular situation that adrenalectomy augmented resistance to a toxic hormone combination [15]

3 DCA elicits the hyalinosis syndrome

As stated in the introduction DCA—especially when given in high doses to sensitized animals—produces the so called hyalinosis syndrome This is mainly characterized by the deposition of hyalin or fibrinoid material in the walls of the arterioles and the renal glomeruli This gradually leads to fatal nephrosclerosis marked poly uria hypertension and a myocarditis similar to that seen in human rheumatic fever The vascular lesions generally resemble those of periarteritis nodosa They are seen quite regularly throughout the arterial tree of the mesentery the pancreas the brain heart and other organs [3 4]

4 LAP produces hyalinosis

All the above mentioned toxic actions of DCA can be reproduced with crude anterior pituitary extracts or LAP The main difference between the syndrome produced by DCA on the one hand and the pituitary preparations on the other is that the latter rarely elicit periarteritis nodosa in the free mesenteric arteries although they do cause such vascular lesions in the heart and sometimes in the pancreas Furthermore LAP elicits a syndrome of splanchnomegaly (marked enlargement of the spleen liver and kidney) this is much less pronounced after DCA administration The splanchnomegaly was attributed to the STH content of the LAP especially since it is regularly accompanied by a marked increase in somatic growth [6,7,3]

5 STH produces hyalinosis

Electrophoretically pure STH reproduces all the above mentioned actions of LAP From this it was concluded that STH is the hitherto unidentified LAP factor which imitates mineralo-corticoid actions Like LAP STH does not tend to produce any mesenteric periarteritis nodosa and causes a pronounced hypertrophy of the adrenals in not hypophysectomized animals [16] However after ablation of the pituitary STH does not tend to cause any adrenal enlargement nor does it produce hyalinosis [11] Apparently endogenous ACTH participates in the production of hyalinosis by STH Perhaps the injection of STH elicits a discharge of ACTH from the animal's own pituitary just as TSH causes an LH discharge and vice versa

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7 Cortisone does not produce hyalinosis

Overdosage with cortisone elicits the same renal changes (essentially characterized by glomerular hyperemia) as ACTH This is in agreement with the generally accepted view that ACTH is primarily

a glucocorticotrophic hormone and that an overdosage with gluco-*corticoids does not result in collagen disease* [3,17]

8 Cortisone aggravates some of the toxic actions of DCA

It is generally known that many of the experimental lesions produced by DCA (as their clinical counterparts such as periarteritis nodosa rheumatic lesions) are prevented by cortisone It is not as generally recognized however that the kidney represents a noteworthy exception

Rats treated simultaneously with large amounts of cortisone and DCA develop even more pronounced nephrosclerosis than those given DCA alone Yet the periarteritis nodosa elicited in the free mesenteric vessels by DCA is completely inhibited by concurrent treatment with cortisone while the lesions in the heart and in the pancreatic vessels may be either inhibited or aggravated The cardiovascular damage caused by mineralo-corticoids may be secondary to changes in the kidney (increased production and/or decreased detoxification of renal pressor substances) In this event the intensity of the cardiovascular lesions could depend upon the relative importance *in each case of the renal damage (which would tend to* increase the severity of the cardiovascular lesions) and the direct action (peripheral protection of the vessels) of cortisone It remains to be explained why the free mesenteric vessels are so *much* more readily protected by cortisone than those in the kidney the heart or the pancreas [3,17]

9 ACTH aggravates some of the toxic actions of LAP

Simultaneous treatment with LAP and ACTH results in a syndrome essentially comparable to that seen after concurrent treatment with DCA and cortisone Here again mesenteric periarteritis nodosa is completely prevented the renal lesions are aggravated and those in the heart pancreas and most other vascular territories are variably influenced depending upon individual factors of susceptibility and other conditions of the experiment [18]

10 Cortisone aggravates some of the toxic actions of LAP

Rats simultaneously treated with LAP and cortisone also respond essentially in the same manner as those concurrently treated *with* DCA and cortisone [19] The observations described in points 8 and 9 are compatible with the view that LAP imitates the DCA like mineralo-corticoid *actions* while *ACTH or cortisone* produce gluco

corticoid effects As noted in experiment no 8 conjoint treatment with mineralo corticoids and gluco-corticoids results in a dissociation of the typical hyalinosis syndrome in that the mesenteric periarteritis nodosa is prevented while the nephrosclerosis and most of the other arterial lesions are aggravated

11 DCA aggravates some of the toxic actions of LAP

Rats simultaneously treated with LAP and DCA exhibit a hyalinosis syndrome essentially similar to that caused by LAP alone or DCA alone (marked nephrosclerosis myocardial and cardiovascular damage but little if any mesenteric periarteritis nodosa) but the evolution of the syndrome *is* much more acute and the severity of the lesions is considerably greater In essence there appears to be a pronounced synergism between the toxic actions of LAP and DCA[8]

12 ACTH aggravates some of the toxic actions of STH

The nephrosclerosis polyuria and myocarditis normally elicited by STH is greatly augmented by simultaneous treatment with ACTH In this respect (and with regard to the other manifestations of the hyalinosis syndrome) the changes produced by conjoint treatment with STH and ACTH are quite comparable to those elicited by LAP and ACTH These observations again agree with the assumption that the active factor in LAP is actually STH[20]

13 Cortisone prevents some of the toxic actions of STH

As we have stated above (point no 10) cortisone aggravates the nephrosclerotic action of LAP Under certain conditions it can also increase the renal damage caused by STH but only if the dosages at which the two hormones are given preclude the production of cortical atrophy by cortisone Usually if large amounts of cortisone are given the adrenal cortex involutes and in that event renal damage is prevented Apparently here the compensatory atrophy of the adrenal cortex protects the kidney from STH just as the cortical atrophy subsequent to hypophysectomy or surgical ablation of the suprarenals If on the other hand the circumstances of the experiment (exposure of the animals to stress inadequate dosage of cortisone) are such that cortical atrophy does not occur then cortisone aggravates the renal damage This is probably due to a peripheral synergism between STH and mineralo corticoids on the one hand and cortisone on the *other*[18] These observations agree with the view that STH is not nephrotoxic in itself and depends for this effect upon

the maintenance of the adrenal cortex either by endogenous or by exogenous ACTH

14 DCA aggravates some of the toxic actions of STH

Simultaneous treatment with STH and DCA *causes particularly* malignant rapidly fatal nephrosclerosis with *polydypsia polyuria* and a marked tendency towards edema formation This could be due to a mere summation between the actions of DCA and of the mineralo corticoids endogenously produced under the influence of STH It may also be the result of an extra adrenal peripheral sensitization by STH of the kidney and connective tissue structures to the toxic actions of the injected DCA [21]

15 DCA aggravates some of the toxic actions of ACTH

The results of conjoint treatment with DCA and ACTH are essentially the same as those obtained by *concurrent administration* of DCA and cortisone except that in *the former case the adrenals* are enlarged in the latter they undergo pronounced *compensatory in*volution In both instances the renal damage is particularly severe while mesenteric periarteritis nodosa does not tend to develop as it would under the influence of DCA alone

GENERAL DISCUSSION

All *these observations are compatible* with the view that *STH* represents that *factor in* LAP *which is responsible* for its mineralo corticoid actions The ability of LAP *or STH to elicit the hyalinosis* syndrome and particularly the characteristic renal *lesions depends* upon the integrity of the adrenal cortex Hence functional inactivation of the adrenal cortex by hypophysectomy or cortisone over dosage as well as surgical removal of the suprarenals prevent these untowards effects of STH Conversely ACTH aggravates them presumably because it increases the amount of STH responsive cortical tissue and at the same time augments the quantity of circulating gluco corticoids which sensitize the kidney to toxic amounts of *mineralo-corticoids* (see below)

The *gluco-corticoids* (*e g cortisone*) counteract some of the toxic manifestations of the *mineralo corticoids* (e g *the development* of mesenteric periarteritis nodosa *the production of a topical irritation* arthritis by the local administration of *irritants to the joint* lesions) On the other hand the renal damage caused by mineralo corticoids is aggravated by gluco-corticoids as shown by the com

bined administration of DCA and cortisone This same aggravation apparently also occurs between the endogenous gluco-corticoids and mineralo-corticoids when rats are simultaneously treated with ACTH and STH

As regards the *mechanisms through which STH could exert its toxic effects* upon the cardiovascular system and the kidney the following possibilities come to mind

1 ACTH is a gluco-corticotrophic hormone (that is one which stimulates the adrenal to produce gluco corticoids) and STH possesses mineralo corticotrophic effects However while ACTH can maintain the integrity of the adrenal cortex (e g even after hypophysectomy) STH fails to do so Hence the latter cannot elicit its potential mineralo-corticotrophic actions after the adrenal cortex has become atrophic due either to cortisone overdosage or to hypophysectomy

2 The secretion of mineralo-corticoids by the adrenal is independent of hypophyseal regulation However due to a peripheral synergism the mineralo-corticoids (e g DCA) sensitize while the gluco-corticoids (e g cortisone) desensitize the tissues to the above mentioned toxic effects of STH

3 STH exerts its effects both through the adrenal cortex and in the periphery It augments mineralo-corticoid secretion and at the same time sensitizes the tissues to the effects of mineralo-corticoids Conversely gluco-corticoids diminish the production of either type of corticoid and concurrently desensitize the tissues to the mineralo corticoid (DCA STH) type of effect

Further experiments are now in progress to explore these three possibilities but at the present time the third hypothesis appears to be most readily compatible with all the known facts

It may be instructive now to attempt a correlation of all these new observations with the previously established facts concerning the mechanism of the hormonal defense reactions to stress during the general adaptation syndrome

As indicated in the figure the *stressor* (trauma infections burns etc) acting directly upon the cells produces *damage* At the same time it also mobilizes for *defense* by evoking a stimulus which induces the anterior pituitary to produce ACTH under certain circumstances it may also cause a discharge of STH The nature of this first mediator between the directly injured organ and the anterior pituitary is not yet known (humoral nervous?) Hence here it is indicated merely by an interrupted line labelled with a question mark *ACTH* induces the adrenal cortex to produce predominantly *gluco corticoid* compounds whose effect upon the response of the

various target organs is generally inhibitory (e g catabolism dimi nution of granuloma formation and of allergic responses) Conversely *STH* enhances a variety of defensive reactions in the target organs (e g anabolism augmentation of granuloma formation and of allergic responses) primarily by stimulating the connective tissue Part of this action is undoubtedly not mediated through the adrenal

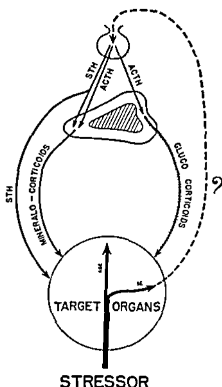


Fig 1 Schematic Diagram illustrating the principal interrelations between the hypophysis the adrenal cortex and the peripheral target organs during the general adaptation syndrome (slightly modified after Selye[7])

cortex but this direct effect sensitizes the connective tissue elements to the (essentially similar) actions of the mineralo corticoids It is probable that STH also acts by increasing the production of mineralo-corticoids However in itself it cannot maintain the cortical cells in a responsive condition hence its corticotrophic effect is dependent upon the simultaneous availability of ACTH In the final analysis the physiologic and pathologic responses of the target organs to stressor agents largely depend upon the balance between the mineralo corticoids and STH on the one hand and ACTH and the gluco-corticoids on the other

SUMMARY

Experiments on female piebald rats revealed that electrophoretically pure somatotrophic hormone (STH) produces nephrosclerosis polyuria myocarditis pancreatic periarteritis nodosa and hypertension These effects correspond to those elicited by an excess of desoxycorticosterone acetate (DCA) or lyophilized anterior pituitary (LAP) It is assumed that STH is the principle responsible for the toxic action of LAP upon the kidney and the cardiovascular system

Large doses of cortisone prevent the cardiovascular and renal damage normally caused by STH overdosage but only if the hormone produces adrenocortical atrophy Complete suprarenalectomy or inactivation of the adrenal cortex by hypophysectomy likewise prevent the above mentioned toxic effects of pure STH and of STH containing LAP

Apparently STH exerts its toxic effects upon the kidney and the cardiovascular system only through (or at least in the presence of) a responsive adrenal cortex

In the production of the so-called diseases of adaptation STH appears to play a role equally as important as that of ACTH The former is responsible for the activity of mineralo corticoids which stimulate defensive granuloma formation while the latter regulates the secretion of gluco-corticoids which inhibit such defense reactions

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DISCUSSION

DR FRANK L ENGEL We have given as much as 5 mgs of Armour s Growth Hormone to rats over a course of thirty or forty days with out ever getting any diuresis Likewise when given with as much as 8 mgs of ACTH we have also gotten no increase in urine volumes I don t know whether we are dealing with a different sort of prep aration of the growth hormone

DR IRBY BUNDING (Armour Laboratories Chicago) Reports from Dr de Bodo of New York University (N Y U) indicate that certain of our (Armour) growth hormone preparations have similarly pro duced diabetes insipidus like effects in the hypophysectomized dog and that these effects can be counteracted by a posterior pituitary principle

DR HANS SELYE In a way Dr Bunding answered Dr Engel s remark because while you didn t get any diabetes insipidus apparently Dr de Bodo did

I think that illustrates very well the importance of what we call conditioning factors in the action of these hormones

I wonder whether you used sensitized animals on a high sodium intake?

DR ENGEL These were animals that were normal on a tube fed high carbohydrate diet We got some diuresis when they developed dia betes but not a very great volume

DR SELYE I think that is true of even the impure LAP preparations unless the sensitized animal had very prolonged treatment with heavy doses necessary

12

The Mechanism of Action of ACTH in Experimental Nephritis Due to Foreign Protein*†‡

Ralph Josiah Wedgwood Clinton Van Zandt Hawn and Charles A Janeway
with the technical assistance of Miriam Ekdahl
THE CHILDREN S MEDICAL CENTER HARVARD MEDICAL SCHOOL BOSTON AND MARY IMOGENE BASSETT HOSPITAL (AFFILIATED WITH COLUMBIA UNIVERSITY) COOPERSTOWN NEW YORK

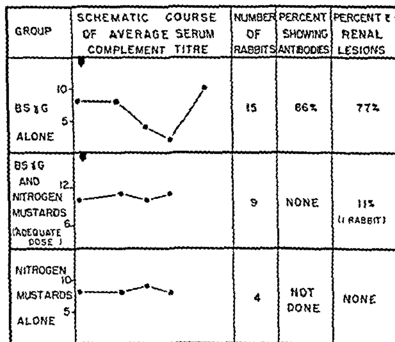
The experimental production of various lesions attributable *to hypersensitivity in rabbits by the injection of foreign proteins has* been demonstrated by many investigators It has been suggested that there exists a similarity between these lesions and those found in serum sickness and possibly the collagen diseases in humans

This laboratory has shown previously that histologic lesions re*sembling those of acute glomerulonephritis in humans attributable* to hypersensitivity can be produced fairly regularly in the rabbit by a single large injection of purified bovine serum gamma globulin In this experimental disease the development of acute lesions is correlated rather closely with certain serological sequences—namely the disappearance of this antigen and a transient depression of the serum complement titre followed by the appearance of circulating antibodies and an increase in serum complement When the formation of antibodies is prevented by adequate doses of X radiation or nitrogen mustards these changes do not occur the complement titre does *not fall and lesions do not develop* [1,2]

Figure 1 shows the composite data from three groups of rabbits and indicates the effect of nitrogen mustards on this experimental disease *The first group of rabbits received a single injection of bo*vine serum gamma globulin By the tenth day following the injection maximal depression of the serum complement titre was evident

* Supported in part by a grant recommended by the Hematology Study Section from the National Institutes of Health U S Public Health Service
† The purified bovine serum *gamma globulin for this study was supplied through* the courtesy of Drs Jules Porsche and James B Lesh by Armour & Company
‡ The ACTH used in this study was supplied through the courtesy of Dr John R Mote by Armour & Company

In eighty six per-cent antibodies could be demonstrated and in seventy seven per-cent definite renal lesions developed The second group received the same injection of gamma globulin and in addition doses of nitrogen mustards adequate to prevent antibody formation and as can be seen no antibodies were formed no depression of the serum complement occurred and with one exception no renal lesions could be found A third group given nitrogen mustards alone showed no serum complement changes and no renal lesions



BS γG 1GM/KG IV AS 16 5% SOLUTION IN 0 3 M GLYCINE

A COMPOSITE CHART FROM DATA OF L SCHWAB ET AL EXP MED 91 505 1950

Fig 1

From these data and the data of Kay[3] and others we postulate the following sequence of events (Fig 2) in the production of these lesions

1 A portion of the circulating antigen is fixed in or on the renal tissue

2 Circulating antigen stimulates the formation of antibodies at the various sites of antibody formation

3 This antibody interacts with the antigen fixed in the kidney

4 This combination possibly as a result of the fixation of complement produces tissue irritation which is manifested by the microscopic lesions

We have found recently that by giving two injections of purified bovine serum gamma globulin ten or eleven days apart (a method similar to that used by More and Waugh[4] but without the unilateral nephrectomy which they performed) we can produce more severe and more easily demonstrable glomerular lesions in about eighty per cent of our rabbits Although demonstration of the appearance of antibodies in these animals is complicated by the second injection of antigen there is the usual close correlation between the rate of disappearance of the antigen the appearance of antibodies the fall in the serum complement titre and the incidence and severity of the renal lesions

The administration of ACTH to rabbits subjected to this double injection technique prevents the development of the renal lesions In figure 3 the data from three groups of rabbits are collected and

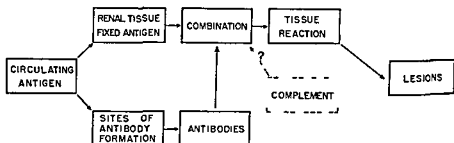


FIG 2

compared In the first group two injections of purified bovine serum gamma globulin were given ten to eleven days apart As can be seen there was a definite fall in the average serum complement titres Of fifteen rabbits in this first group antibodies could be demonstrated in nine (sixty per cent) and definite renal lesions were found in twelve (eighty per-cent) The second group received the same double injection of gamma globulin but in addition four milligrams of ACTH every six hours from the fifth day following the first injection until the time of sacrifice two days after the second injection In this group a similar fall in complement titres was observed and circulating antibodies were demonstrated in six (fifty five per cent) of the eleven rabbits However no renal lesions could be found in any of these rabbits receiving ACTH in direct contrast to the usual correlation of definite renal lesions with the appearance of antibodies and with a depression of the serum complement titre In the third group five rabbits received ACTH alone at the same dosage for the same period of time as the second group As was to be expected none

of these rabbits showed the development of antibodies a fall in serum complement or renal lesions

The dosage of ACTH used was chosen because we found in agreement with other observers that a single injection of four milligrams of ACTH in a two kilogram rabbit produced a statistically significant drop in the absolute circulating lymphocyte count for a period of four to eight hours The action of the hormone was also

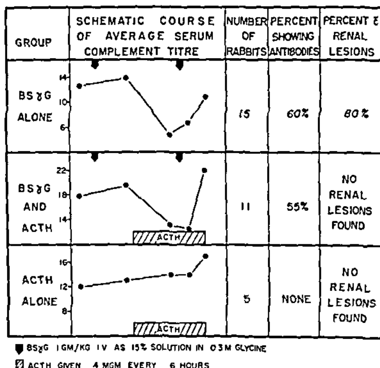


Fig 3

manifest by microscopic changes in the adrenals of all the rabbits receiving the hormone

SUMMARY

The development of glomerulonephritis in rabbits following the injection of bovine serum gamma globulin can be prevented by nitrogen mustards X radiation and ACTH It is our hypothesis (Fig 4) that in the case of nitrogen mustards or X radiation lesions do not develop because the production of antibodies is prevented and con

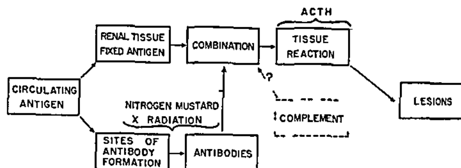


FIG 4

sequently no antigen antibody reaction can occur However in the case of ACTH no lesions appear in spite of the development of anti bodies and in spite of the evidence for the interaction of antigen and antibody as shown by a decrease in the serum complement titre suggesting that the *hormone in some way alters the capacity of the tissues* to react to this usually irritant antigen antibody combination

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DISCUSSION

DR EARL BENDITT (Bobs Roberts Memorial Hospital and University of Chicago The School of Medicine Chicago) It is quite reasonable to suspect that there may be an ACTH induced alteration in the capacity of the tissues to react in the presence of antigen and anti

body On the other hand the possibility exists in the light of recent work that there is a reduction in the quantity of antibody produced Thus Germuth and Ottinger at The National Institutes of Health have shown that under the circumstances of their experiments administration of ACTH to rabbits reduced by about 50 per cent the quantity of anti egg albumin antibody produced

DR BRAM ROSE I should like to ask a question with reference to this paper in view of the experiments of Germuth and Ottinger because I think they showed rather nicely that the production of the Arthus phenomenon in rabbits can be inhibited by the administration of ACTH and that as the antibody production is decreased so does the capacity of the animal to react with the Arthus phenomenon in the skin by the injection of the original antigen namely the horse serum

They also showed that when the animal was under the influence of ACTH the administration of both antigen and antibody resulted in the production of the Arthus phenomenon in the normal way They therefore postulated that the Arthus was dependent on the presence of antibody and that the inhibition of antibody production was the main effect

I wonder therefore if there is any reason to believe that the type of lesion that occurs in the kidney as demonstrated in this experiment is a different type of reaction compared to the Arthus phenomenon which is produced by the injection of foreign serum

DR S HOWARD ARMSTRONG JR I happen to have been quite close to this experimental work in talking with Dr Hawn since it started I do not think that there is any question that the antibody levels that they are getting here are entirely comparable to what they get without ACTH although I realize that in other laboratories an occasional depression of antibody production has been related to ACTH

There are many possible mechanisms about which one can speculate concerning the relation of antigen antibody complexes to the pathogenesis of the histologic alterations seen by this group and by Rich s group in Baltimore In that the data of this paper do not deal with mechanism merely with the fact of blocking discussion is not relevant here

DR GREGORY PINCUS I would like to recall to this audience the observation made by Menkin on the effect of adrenal extract on the inflammatory reaction In those days he did not have pure corticosteroids available He found the action of leukotoxin on the inflammatory agent was inhibited Apparently the chief inhibition occurred

at the capillary The capillary permeability which greatly increases as a result of the inflammatory reaction did not increase

I wonder if we are dealing essentially *with a similar phenomenon* here namely a change in the inflammatory reaction to the antibody

DR RALPH JOSIAH WEDGWOOD There still seems to be some question as to the effect of ACTH on the production of antibodies In our experiments the ACTH was given from the fifth day following the initial injection of antigen in an effort to avoid possible inhibition of antibody production which might occur if the animals were pretreated with the hormone since we felt that by the fifth day circulating antigen should have been in contact with the sites of antibody formation for sufficient time to initiate the usual production of antibodies

The demonstration of the appearance of circulating antibody is *dependent upon the disappearance of circulating antigen* and the inability to demonstrate such antibodies in the presence of circulating antigen does not indicate that such antibodies have not been formed or released The demonstration of circulating antibody in these experiments is hindered by the second injection of antigen given at just about the time one would expect antibodies to appear

In this experimental method we find that those rabbits who do not get ACTH and who show the disappearance of antigen the fall in complement and the appearance of antibodies show renal lesions those who get ACTH show the same sequence of serologic changes but do not get any renal lesions On examination of the serologic changes in individual rabbits one cannot distinguish between the two groups by the fall of the complement titres by the rate of disappearance *of antigen or by the demonstration of the appearance of* antibodies

In the production of these glomerular lesions I do not believe that we are dealing with the usual type of anaphylactic response I am sure that there are at least two types of immunologic reaction responsible for the production of the lesions attributable to hypersensitivity These might be classified as the immediate type which would include the usual anaphylactoid reactions and nephrotoxic nephritis in rats (but not in rabbits) and the reaction that produces the lesions seen *in our laboratory which might be classified as* delayed

Much of the work on the effect of ACTH on the immunologic responses at this time seems to accentuate this hypothesis ACTH seems to have a greater effect experimentally on the delayed type of reaction than on the immediate Our data are certainly consistent with this idea

13

The Relation of the Salivary Sodium Potassium Ratio to Adrenal Cortical Activity*

Thomas F Frawley and George W Thorn

PETER BENT BRIGHAM HOSPITAL AND HARVARD MEDICAL SCHOOL BOSTON

The electrolyte regulating effects of adrenal steroids upon the electrolyte composition of various body fluids and urine are well established Conn[1] has shown that this influence is also exerted upon the electrolyte composition of sweat and that the measurement of sweat sodium and chloride may be used as an index of adrenal cortical activity The present study has been undertaken to ascertain whether adrenal cortical activity may also determine to a large extent the electrolyte composition of saliva In addition to being a readily available secretion there were reasons to suppose that its electrolyte composition might like that of sweat reflect adrenal cortical activity Both sweat and saliva are hypotonic and have as principal ions sodium potassium and chloride In both of these secretions this electrolyte composition is the result of osmotic work by the secreting glands That this secretory work might be under hormonal control has been suggested by Hiki[2] and the calcium concentration of saliva is known to be elevated following overdosage of parathyroid extract

METHODS

Subjects on a moderate but uncontrolled salt intake were studied in the post absorptive state Stimulation of salivary flow was obtained by chewing paraffin and collecting specimens over two consecutive ten minute periods Each saliva specimen was centrifuged and an aliquot made up in distilled water Sodium and potassium were determined by internal standard flame photometry and routine methods were employed for other chemical determinations

The first 10 minute specimen was discarded since it was not considered to be a representative physiological aliquot of the normal sal

* The authors are indebted to William J Reddy A B for technical collaboration during these studies

ivary secretions but rather stored ductal secretions contaminated by oral debris expectorated during the first few minutes of active chewing The observations to be discussed are based on measurements of the sodium and potassium concentration of the second ten minute period specimen

RESULTS

I Variations in the Concentration of the Chemical Constituents of Saliva

Initially several chemical constituents of saliva were measured The concentration of chloride was studied extensively under a variety of conditions and was found to be inconstant and to bear no satisfactory correlation with adrenal cortical function Studies of the concentrations of phosphate and uric acid were equally irrelevant

In comparative studies of normal subjects and patients with Cushing s syndrome or Addison s disease differences were observed particularly between the concentrations of salivary sodium (Fig 1) The normal range of sodium concentrations overlaps as might be predicted with that of treated Addison s disease However, there is no overlap between the high range of untreated Addison s disease or the

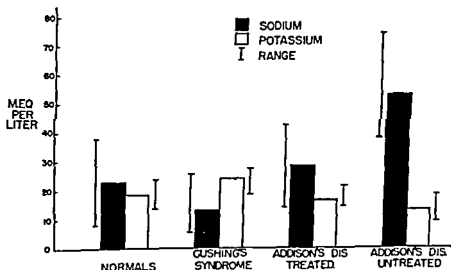


Fig 1

low range of Cushing's syndrome Thus the salivary sodium concentration varies inversely with the state of adrenal cortical function Among all groups the concentration of potassium showed little variability and the ranges of concentration in the groups studied were small but overlapped widely However it is this limited range of potassium change and the small tendency of potassium to vary inversely with sodium which is critical since neither the concentration of sodium or potassium singly appear to separate these groups adequately For these reasons salivary sodium to potassium ratio (Na/K) has been measured in relation to these varying states of adrenal cortical activity

II Variations in the Salivary Na/K Ratio

Normal Subjects In 17 normal subjects the salivary Na/K ratio ranged from 0 33 to 2 1 with a mean of 1 3 (Fig 2) Single saliva Na/K ratios in normal individuals taken at intervals over a period of several months remained relatively constant (see upper part of Fig 5)

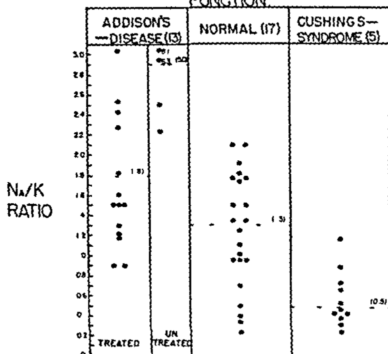


Fig 2

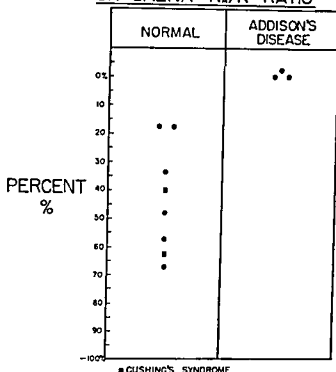


FIG 4

over a period of months (Fig 5) fifteen mg of DCA produced after 24 hours a fall in salivary Na/K ratio from a control level of 0 95 to 0 73 This is approximately a 23 per cent fall and represents the lowest Na/K ratio ever observed in this subject In another subject the same dose of DCA produced a fall in the salivary Na/K ratio of 25 per cent These observations provide definite evidence to the effect that desoxy like steroids alter the electrolyte composition of saliva

SUMMARY AND CONCLUSIONS

The saliva Na/K ratio has been measured in a group of normal subjects and in patients with varying degrees of adrenal cortical activity In general the Na/K ratio has been observed to vary inversely with the state of adrenal cortical activity

It was found to be 1 3 (mean) in the normal group 5 0 (mean) in patients with untreated Addison s disease and 0 5 (mean) in patients with Cushing s syndrome

THE EFFECT OF DESOXYCORTICOSTERONE ACETATE

UPON THE SALIVARY Na/K RATIO

Subject T.F 30 M Normal Subject

Date		Na/K Ratio
3/3/50		1.1
11/8/50		0.95
11/10/50		1.0
12/4/50		0.95
	DCA, 15 mg ,i.m.	
12/5/50		0.73

Fig 5

In patients with markedly lowered serum sodium concentrations the saliva Na/K ratio did not reflect accurately the state of adrenal cortical function

Desoxycorticosterone acetate (15 mg) administered to normal subjects produced a 25 per cent fall in the saliva Na/K ratio

In all patients showing an adequate response to ACTH administration there was a fall in the salivary Na/K ratio of 18 to 68 per cent

The salivary Na/K ratio affords a simple means of following changes in adrenal cortical activity in terms of electrolyte regulation

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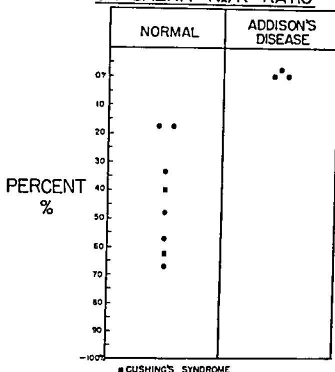


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It was found to be 1 3 (mean) in the normal group 5 0 (mean) in patients with untreated Addison s disease and 0 5 (mean) in patients with Cushing s syndrome

there was a fall in circulating eosinophils irrespective of the dose of ACTH Since these results were in accord with previous work such counts were made only in the first few studies

The expected rise in excretion of 17 ketosteroids and neutral reducing lipids occurred after administration of ACTH The average control excretion of 17 ketosteroids was 0 52 mg/d and that of neutral reducing lipids was 0 17 mg/d The administration of ACTH was followed by a rise in 17 ketosteroids to an average of 1 09 mg/d and a rise of neutral reducing lipids to an average of 0 32 mg/d On con

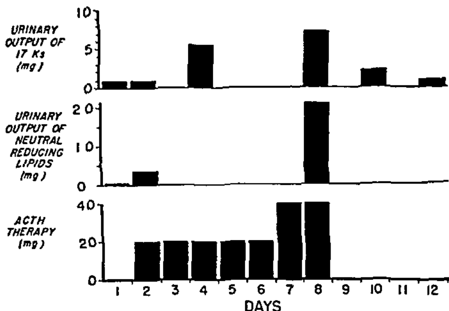


Fig 1

tinued stimulation the 17 ketosteroids rose at times to normal adult female levels and the neutral reducing lipids far exceeded normal adult levels an example of which is shown in Figure 1

The nitrogen excretion in the urine showed a uniform rise when ACTH was administered

The urinary potassium usually increased on the day that ACTH was given This could not be explained by increased protein catabolism using the usual 3/1 ratio of potassium to nitrogen In several cases there was an unexplained secondary rise in urinary potassium 2 to 3 days after ACTH was given This too was associated with increased nitrogen output

The most striking change occurred in the sodium excretion In contrast to most adults there was no restriction of sodium during

It has been suggested that the fetal pituitary is inhibited from producing ACTH by the excess of maternal steroid transmitted across the placental barrier this inhibitory influence is removed after birth[4] The stress of postnatal life then stimulates the pituitary to produce increasing amounts of ACTH until the previously unresponsive adrenal is built up to an efficiently responding organ Up to this hypothetical point the adrenal would be relatively unresponsive to exogenous ACTH just as the adrenal is in some cases of Simmonds disease

In four cases Venning found urinary 17 ketosteroid levels that were high for the first few days of life and then fell to normal infantile levels She has suggested that the early high ketosteroid output may represent maternal hormonal products The later low levels of 17 ketosteroids may well represent nonspecific chromogens The infant under 10 days of age however can excrete a higher level of 17 ketosteroids after ACTH administration as has been demonstrated by Venning in one of her cases and in two of our unpublished cases

The function of the so called fetal cortex has long been a matter of speculation A relationship between it and the cells seen in cases of congenital adrenal hyperplasia (and also with the cells of the normal reticular zone) has been postulated However most of the experimental work has been done in animals and it is very difficult to apply this work to human beings Some insight into the function of the X zone in mice has recently been gained by the work of Jones[7]

The present work was started in an attempt to establish the possible changes in sodium and potassium control in this period of changing corticosteroid production It was soon found however that the observed differences lasted beyond the first few weeks and into the period where Talbot[8] has shown that the excretion of neutral reducing lipids is stable increasing only proportionately to the increasing surface area of the child

In this study determinations of 17 ketosteroids and neutral reducing lipids in the urine were also made in fourteen infants 10 weeks of age or less of which 11 were premature and three were full term infants three weeks of age or less In addition 2 infants 1 six months and 1 eleven months old were studied in a similar manner The intakes were estimated from conventional tables and random samples of the diets were actually analyzed ACTH was administered to 12 patients for one day only in doses of 1 to 10 mgms every 6 hours Two patients received ACTH 4 times a day for periods of 7 and 10 days respectively Cortisone was given to 2 infants one received 25 mgms in a single injection the other 50 mgms Single doses of desoxycorticosterone acetate were given to 4 children

In all infants over one week of age on whom counts were made

sone showed a marked decrease in urinary output of water sodium and potassium This infant was then given ACTH and responded to it in the same manner as the other newborns

In comparison with the infants of the younger age group a study was made on 1 six month old full term infant and 1 eleven month old full term infant Both of these children showed sodium retention when given ACTH for one day Parenthetically mention must be made of a six month old boy who appeared to excrete increased amounts of sodium when given ACTH

Figure 2 shows the electrolyte balances of a 19 day old infant given ACTH of a 6 month old infant given ACTH and of an 18 day old *infant given DCA Each column represents one day Intakes were* plotted down from the zero line and the urinary excretion (blacked in) is plotted up from the intake The resultant white spaces below

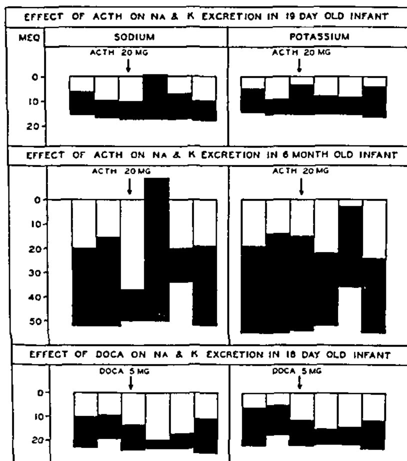


Fig 2

the period of ACTH administration but there was nonetheless a significant increase in the excretion of sodium in the urine on the day following the administration of an adequate dose of ACTH This loss in the urine was often greater than the intake for the day of that ion and varied roughly in ratio to the dose of ACTH It was accompanied by an increase in urinary volume and there was usually a decreased rate of weight gain at this time

In the larger infants who received the larger dose of ACTH there was a suggestion of sodium retention on the day of ACTH administration Even if this were valid it was so slight that it could not account for the increased excretion the next day

Table I gives the average daily intake and urinary excretion of sodium from 10 studies on infants receiving ACTH for one day only

Neither of the two infants who were given ACTH for a week or more showed any signs of escape from the effects of ACTH on sodium

Table I

Rx	*Intake*	*Urinary Excretion*	*Intake—Urinary Output*
0	14 5	6 6	7 9
0	16 8	7 9	8 9
ACTH	17 8	7 5	10 3
0	18 1	16 8	1 3
0	17 6	9 7	7 9
0	18 9	8 7	10 2

excretion This increased excretion continued for 2 to 3 days after ACTH administration was stopped One of these premature infants developed diarrhea while he was receiving ACTH He continued to pour out more sodium in his urine than he took in in spite of the fact that there was probably an increased loss in the stools He did not start to conserve sodium by diminishing urinary output until 3 days after ACTH was stopped Up to this time any additional amounts of parenteral saline received were excreted within 24 hours

When DCA was administered to newborns in doses of 2–5 mgms there was a decreased urinary excretion of sodium and a decreased urinary volume These patients also were given ACTH and showed typical newborn responses DCA did not cause an increased excretion of potassium in these children but instead in several cases there was a decreased excretion coincident with the reduction in urinary volume

Two premature infants were given cortisone When 50 mgms were given to the first infant there was no significant change in electrolyte excretion The second infant who received 25 mgms of corti

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DISCUSSION

DR JOSEPH W JAILER I would like to say a word about the premature human pituitary

We have found using the fall in the eosinophils as a test that the premature at birth up until about the tenth day of life does not respond with a fall in circulating eosinophils to the administration of epinephrine However it does respond of course to the administration of 6 mg of ACTH

Generally speaking we have found that the lighter the infant at birth that is the more premature it is at birth the longer it takes for the pituitary to respond

DR ROBERT KLEIN I think Dr Jailer s interpretation is quite correct Using small doses of ACTH as reported last year we found that during the first week of life the eosinophil response of the newborn infant whether premature or full term was much less striking or much less adequate than it was after that time

We felt that that was probably due to the fact that its adrenals so to speak had not been primed by pituitary ACTH

the zero lines represent positive balances and the blacked in areas above the zero line represent negative balances It must be noted that no allowance has been made for stool or sweat electrolytes

Our patients were predominantly prematures because they were more available for balance studies In our small series there was no difference in response in various weight and maturity groups and previously no difference had been noticed in eosinophil response to ACTH between premature infants and full term infants of the same postnatal age [4] However the abnormal effects of the adrenal on electrolyte excretion may possibly persist longer in premature than in full term infants

Still to be determined are the average time for disappearance of the abnormal sodium excretion whether the ability to retain sodium on ACTH administration is acquired before this abnormal excretion is lost (perhaps greater intakes of sodium might make this more easily demonstrable) and of course whether the increased excretion is due to some abnormal adrenal hormone or to an abnormal balance among the various normal hormones The writer rejects either the possibility that this loss can be due to sweat changes since it is frequently greater than the difference between intake and urinary output in the control periods or the possibility that it can be explained by adrenal exhaustion since it continues and increases with continuing ACTH administration when other hormonal products are still increasing

SUMMARY

There is a period one to two weeks after birth before the adrenal of the newborn has full normal power to respond to ACTH or stress by putting out more corticosteroids Up to this time the basal output of these hormones is also low This is a quantitative change only and even in this period the adrenal can respond to ACTH After this period the response of the infantile adrenal as measured by steroid excretion is relatively as good or better than that of the adult provided that the dosage is not scaled down in absolute proportion to size

On the other hand at least one to two months are required before stimulation of the adrenal of the infant will produce significant sodium retention Adrenal stimulation of the newborn infant before this produces a loss of sodium that can have serious effect if the stimulation is continued

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A reduction in the venous hematocrit occurred in all patients ranging from 3% to 26% Although the renal plasma flow as measured by the clearance of para aminohippurate increased by from 17% to 37% in the 3 patients in whom it was measured the effective renal blood flow as calculated from the venous hematocrit and the renal plasma flow was not significantly altered

The glomerular filtration rate as measured by the clearance of inulin increased in all patients by from 20% to 43% the increases being most marked in the patients receiving ACTH The filtration fraction was not significantly altered

In this group of patients the mean control clearance of endogenous creatinine was 115% of the clearance of inulin Following the administration of ACTH or cortisone the creatinine clearance was only 94% of the clearance of inulin This change in the ratio of the clearances of creatinine and inulin was statistically significant for the entire group and in three of the four patients taken individually Assuming that a ratio of creatinine and inulin clearances which is greater than 1 0 is the result of both filtration and tubular secretion of creatinine an increase in filtration alone would be expected to lower the ratio toward 1 0 However the increases in the filtration rates in these patients were not sufficient in themselves to account for the reductions in the clearance ratios which occurred It is therefore concluded that the administration of ACTH or cortisone induced an alteration in the excretion of creatinine and inulin or both Similar findings have been observed in patients with Addison s disease treated with cortisone[1]

The factors which regulate the rate of excretion of uric acid and electrolytes are not known with certainty but may include their serum concentration the rate of glomerular filtration or independent changes in renal tubular metabolism Where altered excretion is the result of a change in the production destruction or release of a substance the amount presented to the kidney as indicated by the product of the plasma concentration and the glomerular filtration rate (filtered load) should be changed in a similar direction

In most instances changes in the rate of excretion of a substance during the administration of ACTH or cortisone occurred despite contrary changes in its plasma concentration and in its rate of filtration or presentation to the kidney indicating the primary role of alterations in tubular transport In those instances in which the rates of excretion and of filtration of a substance changed in similar directions an alteration in the ratio of the rate of excretion to the rate of filtration is also considered to be indicative of a change in tubular activity with regard to that substance

15

The Effects of ACTH and Cortisone on the Renal Tubular Transport of Uric Acid, Phosphorus and Electrolytes in Patients with Normal Renal and Adrenal Function*†

Sidney H Ingbar Edward H Kass, Charles H Burnett, Arnold S Relman Belton A Burrows, and John H Sisson

THORNDIKE MEMORIAL LABORATORY BOSTON CITY HOSPITAL HARVARD MEDICAL SCHOOL THE EVANS MEMORIAL MASSACHUSETTS MEMORIAL HOSPITALS AND BOSTON UNIVERSITY SCHOOL OF MEDICINE BOSTON

The data from earlier investigations with ACTH and cortisone together with observations made in patients with pneumonia treated with ACTH suggested that the adrenal steroids may influence renal function The present study was undertaken in an attempt to determine the nature of such effects upon the normally functioning kidney and the extent to which the kidney participates in the over all metabolic response to these hormones The data obtained could also serve as a baseline for investigations of the effects of ACTH and cortisone upon disordered renal function

Four patients were studied their renal function was found to be within normal limits by the usual clearance techniques Two of these patients were then given ACTH and two were given cortisone Clearance measurements were repeated on the last day of administration of hormone under conditions as nearly identical to those of the control tests as possible Increasing doses of the hormones were given so that in the 24 hours prior to their respective tests the patients who received ACTH were given respectively 275 mg and 430 mg and those who were given cortisone received 600 mg In an effort to produce maximum functional alterations the doses administered were larger than those usually employed since small but significant changes in renal function may be obscured by the inaccuracies of the clearance technique

* This work was aided by a grant from the United States Public Health Service and part of it was done under contract with the Army Medical Research and Development Board

† The ACTH was supplied by Dr John R Mote of the Armour Laboratories

fer of uric acid under the influence of adrenal steroids are in agreement with the conclusion of investigators using other techniques that the uricosuric effect of ACTH and cortisone is renal in origin [2,5]

Phosphorus (Fig 2)

Phosphorus excretion increased in all patients despite diminished or constant rates of filtration of phosphorus The ratio of excreted to filtered phosphorus rose by at least 100% in all patients and by as much as 300% in patient D S These data demonstrate that altered renal tubular transfer of phosphorus is at least in part responsible for the increased phosphorus excretion which may follow the administration of ACTH or cortisone and may account for the losses of phos

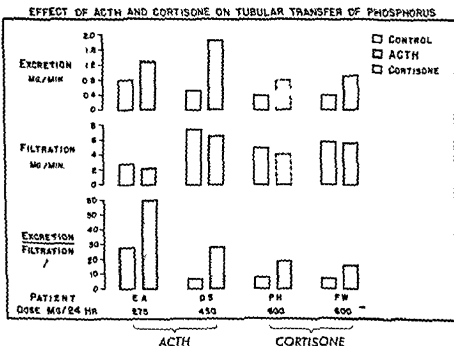


Fig 2

phorus which could not be accounted for by losses of nitrogen or calcium [4,5]

The administration of para aminohippurate is known to increase phosphorus excretion [6] and loading with sodium para aminohippurate resulted in increased phosphorus excretion in all 4 patients both prior to and during the administration of ACTH or cortisone The increments in phosphorus excretion resulting from loading with sodium para aminohippurate while the patients were receiving ACTH,

Uric Acid (Fig 1)

In the two patients who received ACTH marked increases in the rate of excretion of uric acid occurred despite decreased serum concentrations which resulted in significant decreases in the rate of its filtration The percentage excretion of filtered uric acid increased markedly—by 200% in one instance and 100% in the other

In one patient who received cortisone collection errors may have occurred (indicated in the charts by dotted lines) and may have invalidated measurements of absolute rates of excretion and filtration but the ratio of excretion to filtration need not have been altered by

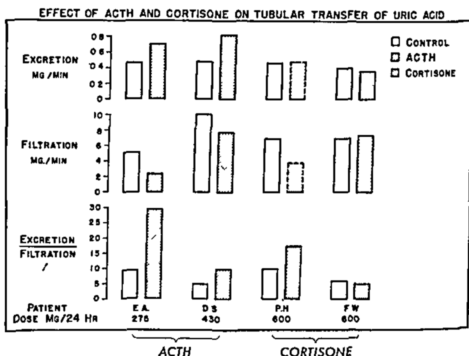


Fig 1

collection errors This demonstrated an increase of 80% during treatment with cortisone

It is of interest that in patient F W who received cortisone no change in tubular activity with regard to uric acid was demonstrated Excretion and filtration were not significantly changed nor was the percentage excretion of filtered uric acid In this patient daily estimations of uric acid excretion revealed no change during cortisone therapy

These findings which demonstrate an alteration of tubular trans

Potassium (Fig 4)

Only in patient P H who received cortisone were potassium excretion and the ratio of excreted to filtered potassium increased prior to loading with sodium para aminohippurate In the remaining 3 patients potassium excretion and the ratio of potassium excretion to potassium filtration were actually decreased

Loading with sodium para aminohippurate prior to the administration of hormone resulted in little or no change in the absolute or percentage excretion of filtered potassium During the period of hormone administration however loading with this material resulted in markedly augmented absolute and percentage excretion of filtered *potassium*

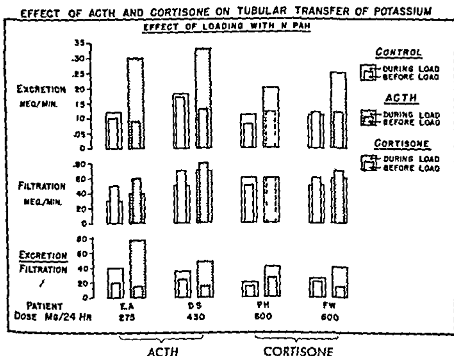


Fig 4

It is noteworthy that the tendency to excrete more potassium during the administration of ACTH or cortisone was not apparent in 3 patients until loading with sodium para aminohippurate was performed Balance data in patient P H reveal that the greatest negative potassium balance which this patient displayed during the administration of cortisone occurred on days in which sodium para aminohippurate was administered In patient F W the only days on which negative potassium balance occurred were those on which

were greater than they had been during the control tests Cortisone however did not produce this effect

Sodium (Fig 3)

In 3 of 4 patients prior to loading with sodium para aminohippurate sodium excretion decreased under the influence of ACTH or cortisone In patient D S sodium excretion was increased However he was the only patient who was given ACTH intravenously during the clearance tests and since this induced a marked antidiuresis the natriuresis which he displayed may represent the activity of contaminating posterior pituitary hormone which was present in the amount of 1 0 units of pressor per injection These decreases in excretion oc

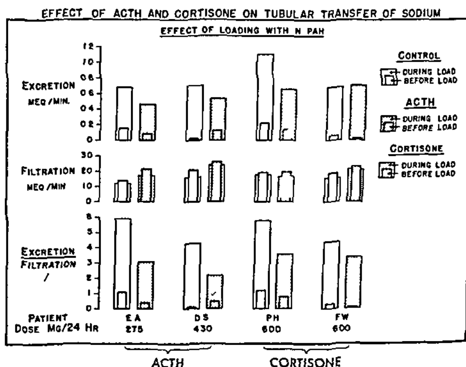


FIG 3

curred despite increased or constant rates of sodium filtration Again except in the case of patient D S the ratio of excreted to filtered sodium decreased significantly during hormone therapy

In all instances the administration of loads of sodium para aminohippurate incident to the determination of T_mPAH resulted in large increases in the excretion of sodium During the period of hormone administration however the increases in both the absolute and percentage excretion of filtered sodium during loading periods were less than they had been during control tests

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DISCUSSION

DR FREDERICK C BARTTER (Massachusetts General Hospital and Harvard Medical School Boston) In a large number of studies in which we have observed this increased excretion of potassium on the first day of ACTH therapy there has not been a substantial retention of sodium until the second day This is hard to reconcile with a cation exchange hypothesis

DR JACK METCOFF I should like to reaffirm Dr Ingbar s observations and in addition ask two general questions

First I wonder whether the excretion filtration ratio is entirely valid in terms of electrolyte clearance Might it not be invalidated by tubular electrolyte secretion?

Second the pK of PAH (para aminohippurate) as a load is about 3 8 which simply means that in the presence of an acid urine the amount of anion solute requiring obligatory cation excretion is variable This feature has bothered us a great deal Para aminohippurate as well as other loading solutes initiates a very large excretion of potassium independently of the administration of ACTH

We studied a normal patient who was loaded initially with a large amount of Na thiosulfate subsequently with very small quantities of sodium para aminohippurate during the next interval and finally with very large quantities of Na PAH (sodium para amino hippurate) It may be noted that there was no change or very little change in serum water concentrations of sodium or potassium A fairly large and prompt increment in sodium excretion was noted There was a very prompt initial rise in potassium excretion as Dr

loading with sodium para aminohippurate was performed During the administration of this material the excretion of large quantities of the nonreabsorbable para aminohippurate anion may have forced the excretion of large quantities of cation In the presence of increased sodium reabsorption and sodium retention potassium appears to have been substituted for sodium

It appears possible therefore that the effect of the adrenal steroids on potassium excretion may result from augmented sodium reabsorption and may depend upon the nature and quantity of anion claiming excretion An intratubular exchange of potassium for sodium may therefore explain the losses of potassium in excess of or in the absence of losses of nitrogen which may occur when ACTH or cortisone is administered[3 4]

It is of interest to note that such excessive losses of potassium are frequently most marked on the first day of hormone administration and subsequently diminish or disappear by the third or fourth day This sequence of events suggests that the renal mechanism for the formation of ammonia which takes 3 to 4 days to become fully activated may provide increasing amounts of cation and may thus spare potassium That this may be a phenomenon general to diverse states producing sodium retention is indicated by observations that the administration of sodium loads to patients with the nephrotic syndrome results in the excretion of large amounts of potassium and that mercurial diuretics when given to patients with sodium retention of cardiac or hepatic origin may result in marked increases in potassium excretion

CONCLUSIONS

1 The administration of ACTH or cortisone may induce alterations in normal renal function as evidenced by changes in the renal plasma flow glomerular filtration rate and the ratio of the clearances of creatinine and inulin

2 Changes in the renal tubular transport of uric acid and electrolytes occur during the administration of ACTH and cortisone and contribute significantly to the over all metabolic response to these agents

3 The effect of the adrenal steroids on potassium excretion may result from augmented sodium reabsorption and may depend on the nature and quantity of anion claiming excretion

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16

Observations on the Relation of Renal Function Changes to the Electrolyte and Glycosuric Effects of ACTH*

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NEW YORK

Administration of ACTH often causes sodium chloride and water retention potassium loss and at times glycosuria particularly at high dosage levels These effects are supposedly the result of increased amounts of adrenal cortical hormones acting on the renal tubule cells However evaluation of effects on the renal tubules is difficult unless glomerular filtration rate is also assessed The present paper represents a study of the effects of ACTH on normal kidney function and of the relation of changes in renal function to the electrolyte and glycosuric effects of the hormone Seven subjects with normal kidneys were selected for study

The effect of ACTH on glomerular filtration rate and renal plasma flow is illustrated in Figure 1 Seventy five to 200 mg ACTH were administered daily in divided doses for 8 to 22 days Filtration rate indicated by the solid lines increased slightly in the first 3 subjects given ACTH while very large increases were noted in subjects 4 5 and 6 The increases in filtration rate in these 6 patients ranged between 13 and 114 ml /minute with an average of 54 ml /minute Patient 7 had an unaccountably high control filtration rate and was the only one to exhibit a decrease during ACTH administration Renal plasma flow indicated by the dotted lines was measured in 5 of the subjects and showed slight to moderate decreases in 4 The renal plasma flow increased in subject 4 but not as much as did the filtration rate Studies performed 4 to 13 days after ACTH administration was terminated in subjects 3 4 and 5 indicated return of renal functions to the pretherapy range

As a result of the changes in filtration rate and renal plasma flow the filtration fraction increased in 4 of the 5 subjects number 7 being

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* This work was supported in part by a grant from the Life Insurance Medical Research Fund The ACTH was generously donated by the Armour Laboratories

Ingbar told us If followed over a period of about an hour and eighty minutes potassium excretion gradually diminishes to its control level

In a patient having the edema of the nephrotic syndrome quite different manifestations were noted With infusion of sodium as the loading solute a very large excretion of potassium was initiated Little augmentation of sodium excretion occurred The excretion of potassium was greater than could be accounted for on the basis of filtered potassium These observations suggest that there is something peculiar about tubular potassium transfer which at least in diseased individuals and temporarily in the normal may be quite independent of obvious ACTH or cortisone effects

DR SIDNEY H INGBAR I am very much interested in the observations of Dr Bartter However I believe that such findings are unusual in that *potassium loss most frequently occurs early and is accompanied* by sodium retention

We realize that changes in the ratio of excreted to filtered material are not conclusive evidence of a change in tubular function per se and therefore we feel more assured in our conclusions because in many instances in these patients not merely did the ratio change but the absolute rates of excretion and filtration were altered by ACTH or cortisone in opposite directions

As Dr Metcoff pointed out para amino hippuric acid is a rather strong acid and has a low pK The lowest urine pH values which we observed in these patients were about 4 5–4 8 At such pH values only a small percentage of the PAH excreted could have been excreted with hydrogen ion Despite the high rate of excretion of PAH in *these patients the apparent cation deficit in the urines of these pa*tients could not be explained on the basis of hydrogen ion excretion in relation with PAH It may however have represented the excretion of titrable acid in association with phosphate and also perhaps ammonia In addition in patients with nephrotic salt retention administration of an alkaline salt $NaHCO_3$ has been demonstrated by Burnett and his collaborators to result in marked increases in potassium excretion similar to those seen during the administration of NaPAH in these patients receiving ACTH or cortisone

(Note see additional discussion following Observations on the Relation of Renal Function Changes to the Electrolyte and Glycosuric Effects of ACTH p 141)

cosuria Nevertheless it can be seen how an increased filtered load of glucose resulting from a greatly enhanced rate of glomerular filtration during ACTH administration could exceed Tm_G and thus result in glycosuria Impairment of carbohydrate tolerance by ACTH would further contribute to glycosuria produced by this mechanism

This probable mechanism does not deny the possibility of a renal glycosuria due to an action of hormones on the renal tubule cells Nevertheless when changes in the ratio of filtration rate to Tm_G as great as those in subject 6 can occur it is imperative to measure filtra

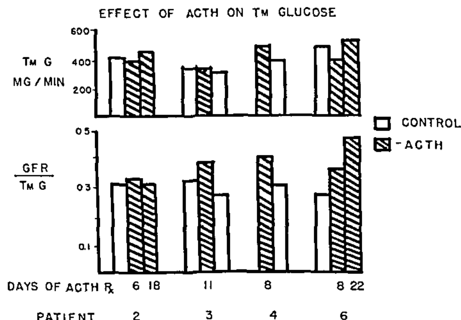


Fig 2

tion rate or to demonstrate independence of glycosuria from carbohydrate intake if true renal glycosuria is to be established Kendrick et al [8] have noted increases in filtration rate with little or no change in Tm_G in patients with various diseases treated with ACTH and cortisone

Retention of water and salt is one of the most characteristic effects of large dose ACTH administration The daily sodium *excretion in* each of the 3 subjects shown on Figure 3 promptly fell to low levels when ACTH administration was begun This undoubtedly was a result of an hormonal effect on the tubule cells for no decrease in glomerular filtration rate (indicated by the numbers *in* boxes with arrows) or in serum sodium level occurred In addition increased so

the only exception The increased filtration fraction together with the rapidity with which changes occurred either on ACTH administration or withdrawal suggest that the observed alterations in filtration rate and renal plasma flow were the result of functional rather than morphological effects Construction of the efferent arterioles of the glomeruli perhaps associated with dilatation of the efferents is the most ready explanation

Berliner[2] observed increased filtration fractions in patients given 40 to 50 mg ACTH daily in divided doses for 7 days These patients

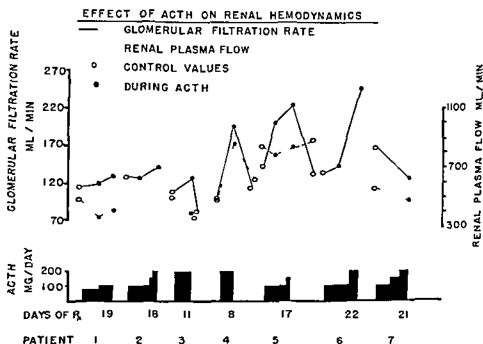


Fig 1

exhibited decreased renal plasma flow but no change in filtration rate Ingbar Relman Burrows Kass Sisson and Burnett[3] however observed increases in both glomerular filtration rate and renal plasma flow when large doses of ACTH (up to 400 mg daily) were given for several days

The maximum ability of the renal tubules to reabsorb glucose (Tm_G) was measured in 4 subjects illustrated in Figure 2 No changes in Tm_G were produced by ACTH in 2 of the subjects while variable changes of doubtful significance were noted in the other 2 However because the filtration rate usually increased during ACTH administration the ratio of filtration rate to Tm_G increased in 3 of the 4 subjects Unfortunately none of these subjects developed sustained gly

cosuria Nevertheless it can be seen how an increased filtered load of glucose resulting from a greatly enhanced rate of glomerular filtration during ACTH administration could exceed Tm_G and thus result in glycosuria Impairment of carbohydrate tolerance by ACTH would further contribute to glycosuria produced by this mechanism

This probable mechanism does not deny the possibility of a renal glycosuria due to an action of hormones on the renal tubule cells Nevertheless when changes in the ratio of filtration rate to Tm_G as great as those in subject 6 can occur it is imperative to measure filtra

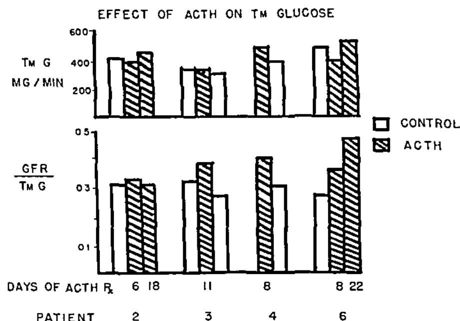


Fig 2

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Retention of water and salt is one of the most characteristic effects of large dose ACTH administration The daily sodium excretion in each of the 3 subjects shown on Figure 3 promptly fell to low levels when ACTH administration was begun This undoubtedly was a result of an hormonal effect on the tubule cells for no decrease in glomerular filtration rate (indicated by the numbers in boxes with arrows) or in serum sodium level occurred In addition increased so

dium excretion developed promptly when ACTH was decreased or stopped (subjects 1 and 2) Note however that sodium excretion soon began to increase in spite of continued ACTH administration even when the dose was increased to 200 mg daily (subjects 2 and 3) Sometime between the 10th and 15th days of ACTH dosage sodium *excretion had returned more or less to the control range* At this point the weight gain ceased Note also that the filtration rate was increased at about the time that sodium excretion returned to its control level

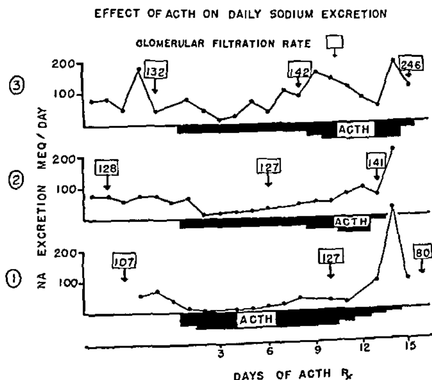


FIG 3

Similar studies in 3 other subjects are shown in Figure 4 Sodium excretion in subject 7 exhibited no consistent response to ACTH although he gained considerable weight Sodium excretion however fell to very low levels in subjects 4 and 5 Note the rather dramatic increases in sodium excretion that occurred in subjects 4 and 5 while *still receiving ACTH* This was accompanied by diuresis and loss of several pounds in weight The filtration rate in subject 4 had increased considerably some days before the diuresis developed and had increased slightly in subject 5 about the time of diuresis The subjects (3 4 5 6) *with the greatest increases in filtration rate* (20 81 81 and 114 ml /min) exhibited the smallest weight gains (3 lbs

or less) at the time of their maximum filtration rates In contrast sub jects 1 and 2 with relatively small increases in filtration rate (14 and 15 ml /min) and subject 7 whose rate decreased during ACTH ad ministration had gained 5 or more pounds at the time of their maxi mum changes in renal function

In all these studies chloride excretion closely paralleled sodium excretion Although the plasma potassium level fell during ACTH

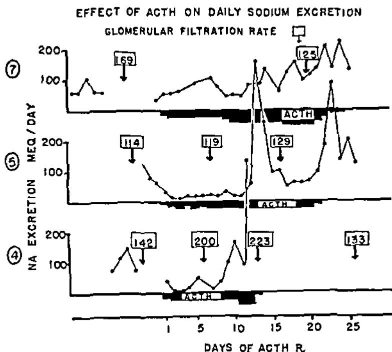


Fig 4

dosage in each subject daily potassium excretion did not change in 2 increased only slightly in 2 and increased significantly in 2

SUMMARY

In summary ACTH administered in daily doses of 75 to 200 mg to subjects with normal kidneys resulted in moderately to greatly in creased glomerular filtration rates reduced renal plasma flows and increased filtration fractions

The maximum ability of the tubules to reabsorb glucose showed no consistent response to ACTH although the ratio of filtration rate to Tm_G increased in 3 of 4 subjects

Sodium chloride and water retention induced by ACTH may be largely the result of hormonal action on the renal tubules

The secondary rise in sodium excretion during continued ACTH administration however could in part be due to the increased glomerular filtration rate

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DISCUSSION

DR HANS SELYE From the morphological point of view I could add to Dr Earle s comments an effect which I think fits in very nicely with his renal function tests

The glomeruli of animals treated with large doses of ACTH are very hyperemic because the glomerular loops are extremely dilated This is an extremely striking change which we noted in the first animals treated with cortisone (Selye STRESS Acta Publ Montreal *1950)* it is to my knowledge not duplicated by any other experimental procedure

Just what causes this dilatation we do not know A constriction of the afferent and dilation of the afferent arterioles could explain it but we have no serial sections to actually prove this

DR W D ROBINSON (University Hospital and University of Michigan Medical School Ann Arbor) In our studies it has appeared that at the time of sodium escape on about the fifth day of continued ACTH dosage there has been a coincident return of urinary potassium excretion from elevated to approximately base line values I would like to ask Dr Earle if he has made similar observations and whether such observations fit with the concept that sodium escape is due to an increase in glomerular filtration rate

DR JOHN TALBOTT (Buffalo General Hospital and University of Buffalo School of Medicine Buffalo) I d like to inquire regarding the

time of administration of ACTH in relation to the renal blood flow and the glomerular filtration studies

This question would apply equally to the previous paper

I am aware of Dr Selye s anatomical changes but one wonders whether or not—if this is a chemical effect—whether it is the maximum chemical effect or whether some of the differences in the flow studies are related to the time of administration

DR MARVIN F LEVITT (Mount Sinai Hospital New York City) We have performed similar experiments and likewise have noted that the glomerular filtration rate and filtration fraction have progressively increased during cortisone or ACTH therapy reaching their peak at about the eighth or ninth day of treatment

However the patients were all maintained on a salt free rice diet and despite the augmentation of the filtration rate and the filtered sodium load there was only a very mild increase in sodium excretion

DR ARTHUR J MERRILL I should like to ask if the tubular excretion of para aminohippurate was studied in these renal plasma flow experiments?

DR IRVINE MCQUARRIE (University Hospital and University of Minnesota Medical School Minneapolis) I should like to ask whether these workers or any others here have studied the decrease or increase in urinary antidiuretic substances such as pitressin during the course of administration of ACTH We see these escapes which suggest that some antagonistic mechanism may be coming into play

DR JOHN LUETSCHER JR (Stanford University School of Medicine San Francisco) In answer to that specific question there are at least two factors concerned with the antidiuresis which is seen early in the treatment of nephrosis with either ACTH or cortisone

In the patient who is treated with ACTH there is usually an early water retention which may coincide with the retention of sodium However an equal retention of water may be seen in patients who are putting out no sodium in the urine before treatment such as the patient with the nephrotic syndrome In this case the retention of water can not be secondary to the retention of sodium

Furthermore water retention may occur under treatment with cortisone just as it occurs with ACTH which I believe rules out the possibility that it is due only to a posterior pituitary contaminant of the administered drug

Now it is possible to demonstrate posterior pituitary activity—or something with a comparable antidiuretic action—in the serum

of some patients with the nephrotic syndrome during administration of cortisone or ACTH After the first week of treatment the antidiuretic material may disappear from the serum and diuresis may ensue

The demonstration of similar antidiuretic activity in urine is possible but measurement is difficult because of the small quantities and the errors introduced by concentration of urine

DR DAVID P EARLE JR Dr McQuarrie s question has been answered for me by Dr Luetscher I was going to have to say that I couldn t answer that question because we had no observations

It is pleasing to learn that Dr Selye s anatomical observations fit in with the functional data In relation to that I might comment that another group has measured filtration rate and renal plasma flow in normal patients treated with much smaller doses of ACTH Berliner has administered 40 to 50 milligrams of ACTH in divided doses for a week to a number of patients and observed no change in filtration rate but considerable decreases in renal plasma flow

In relation to the question on sodium escape and its relation to potassium we couldn t tell very much from our data because the changes in potassium excretion were so small

In reply to Dr Merrill we have not measured the extraction ratio for PAH in these patients To tie the matter down that should be done However we work at very low plasma levels of PAH and hope that it s all right

In reply to the question about the relation between the time of administration of ACTH to the time of measurements of renal function the patients received their morning dose but allowing time for equilibration and so on generally three or four hours elapsed after the last dose of ACTH before the functions were measured

Berliner using his smaller doses examined this a little bit more thoroughly and found that the changes in sodium and potassium excretion occurred quite early and before there were any changes in renal plasma flow He examined some of his patients the day after stopping dosage and observed return of renal plasma flow to normal within 24 hours

In relation to the effect of ACTH on early potassium excretion I think I m quoting Dr Berliner correctly when I say that he felt that occurred within 15 or 20 minutes of ACTH administration and was quite *transient* He further found that pitressin more or less in the same amount that is supposedly present in ACTH produced the same effect

I might make one additional comment Several of the previous

speakers and discussors mentioned the effect of loading with sodium PAH on electrolyte excretion

We have studied this in approximately 25 patients with normal and diseased kidneys and have been impressed by the very variable effects Sometimes sodium excretion goes up sometimes potassium excretion goes up and sometimes both increase Sometimes neither electrolyte changes One thing that seemed to be fairly consistent was that chloride excretion increased relatively less than did either sodium or potassium excretion An actual decrease in chloride excretion was not at all uncommon

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Effects of ACTH on the Pathologic Physiology and Clinical Course of the Nephrotic Syndrome in Children*†

*Jack Metcoff, Weston Kelsey,‡ C Phillips Rance** and Charles A Janeway*

CHILDREN S MEDICAL CENTER AND HARVARD MEDICAL SCHOOL BOSTON

This preliminary report concerns observations on renal *mechanisms associated with diuresis in the edematous nephrotic* child subjected to ACTH therapy It will suggest that altered renal hemodynamics may be the result rather than the cause of favorable response

Thirty five of the ninety children with the nephrotic syndrome studied in our clinic during the past year have received ACTH therapy The usual daily dose was 150 to 200 mg per sq M surface area for 10 days Diuresis was induced in two thirds of the first thirty patients treated (Fig 1) Diuresis has been observed during continued administration of ACTH but commonly begins twenty four hours after its withdrawal Maximal diuresis is usually attained within six to nine days Thirteen children who diuresed have exhibited sustained remissions for a two to ten months observation period with a recurrence of edema in the remaining nine patients

Thirteen of the thirty five treated children developed hypo tonicity (260–270m osmol/L serum H_2O) during therapy Hypotonicity is occasionally observed in untreated nephrotics Serum osmolarity was estimated by addition of values for all electrolytes (except sulfates organic acids and urea) determined by direct measurement An approximation of osmolarity of the extracellular water compartment may be estimated from the relationship 2(Na x 1 04 + 10) Hyponatremia occurred as early as the second day after institution of therapy Hyponatremia and hypotonicity appeared to be di

The Armour Laboratories kindly supplied some of the ACTH used

† Supported in part by grants from the National Institutes of Health U S Public Health Service and Mead Johnson & Co

‡ Post Doctorate Research Fellow National Institutes of Health U.S P H.S on leave of absence from The Bowman Gray Medical School Winston Salem N C

Kellogg Foundation Fellow

rectly related to use of ACTH and could not be entirely accounted for on the basis of weight gain resulting from unrestricted fluid intake This is suggested by Figure 2 which depicts the results of a protracted metabolic balance study during successive courses of ACTH in a 3-10/12 year nephrotic child. The upper portion of the chart indicates the daily variation in some of the serum electrolytes The lower part of the chart represents the algebraic cumulative balance data for sodium potassium, and chloride. All data contributing to balance—intake and excreta—were derived by direct analysis

NEPHROTIC SYNDROME
CLINICAL RESPONSE TO ACTH THERAPY
I. DIURESIS

RESPONSE	PATIENTS	COURSES
DIURESIS	22	24
NO DIURESIS*	8	12
TOTAL	30	36

*3 DEATHS OCCURRED DURING THERAPY 10/50

Fig 1

In this chart, the change in body weight has been plotted in terms of chloride in the ECW thereby indicating weight changes attributable to dilution or contraction of the ECW compartment. The peak weight increments appear to coincide with maximal hypotonicity Calculation of electrolyte and water shifts based on the Darrow arithmetic however indicate that the migration of sodium into the intracellular water compartment significantly contributed to hyponatremia. The mechanism of this apparent shift remains undetermined.

Three deaths resulted from therapy One death occurred suddenly on the tenth day of therapy and was associated with hypo-

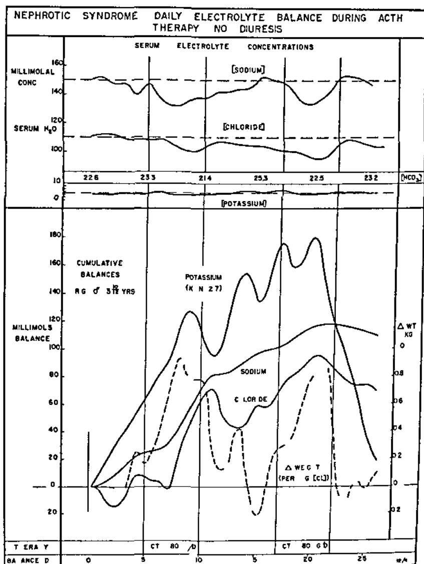


Fig 2

tonicity hyperkalemia and convulsions Hypertension had been noted intermittently during therapy Post mortem revealed only typical nephrotic kidneys In another instance hypertension (140/100) was present prior to therapy Convulsions coma hypotonicity and azotemia developed on the sixth day of ACTH *without change in* blood pressure Hypotonicity and azotemia were promptly repaired Blood pressure increased sporadically Coma persisted until death occurred approximately six weeks later In the remaining fatal case hyponatremia was noted and repaired during the period of ACTH administration Aureomycin was given in full doses before during

and after therapy Some vomiting occurred After abortive diuresis pneumonitis peritonitis and C Coli bacteremia were discovered on the fourth post ACTH day Death resulted from subsequent complications

Either bronchiolitis pneumonitis peritonitis and/or bacteremia have occurred in four children during ACTH administration although full therapeutic doses of aureomycin terramycin or penicillin and streptomycin were used prophylactically These children recovered following cessation of ACTH and continued antibiotic therapy

Detailed renal function studies in six children before therapy and again after diuresis revealed striking increases in filtration fraction defined as the ration of glomerular filtration rate (inulin) to renal plasma flow (PAH) (Fig 3) The increased filtration fraction

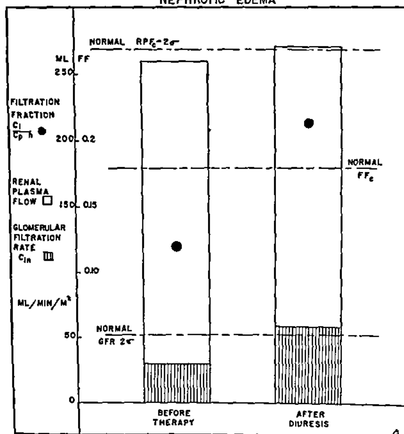


FIG 3

usually resulted from an absolute augmentation of glomerular filtration Improved filtration was associated with a marked decrease in extracellular water (measured as volume of distribution of thiosulfate) Satisfactory measurements of plasma volume using the dye T 1824 and an acetone extraction technique[1] have been obtained in five instances before and after diuresis Plasma volumes were increased in two patients following diuresis decreased in two and no change occurred in the remaining patient It seems unlikely therefore that reciprocal change in volume of either interstitial or plasma compartments is essential to initiate improved filtration

Hemodynamic factors initiating alteration in effective renal arteriolar resistance might be related to the observed improvement of filtration In (Fig 4) spontaneous and ACTH induced diuresis observed in the same patient and expressed as change of body weight are compared with measured changes in filtration and calculated changes in renal arteriolar resistance The abscissa indicates the period over which measurements were made and the arrows in the mid portion of the chart refer to the specific times at which these measurements were obtained The depicted relationships represent a typical pattern associated with edema and diuresis

It will be noted that values for glomerular filtration and filtration fraction were inversely related to changes in body weight The improved filtration following diuresis conforms with the hypothesis that depressed renal function during the early phases of the nephrotic syndrome in children is associated with reversible physiologic rather than irreversible morphologic impairment[1]

Improved filtration indicates increased effective filtration pressure The effective filtration pressure is the result of hydrostatic pressure exerted on the glomerular membrane and the forces resisting filtration The latter include the oncotic pressure of plasma and the subcapsular and/or renal interstitial pressure The lowermost portion of the chart is designed to show the changes in arteriolar resistance relative to normal Arteriolar resistances which were estimated by application of Lamport s formulae[2] Such calculation undoubtedly affords a very crude approximation of the effective resistances and pressures associated with glomerular filtration In this instance augmented filtration seems characterized by relatively decreased afferent and increased efferent arteriolar resistance A similar pattern characterizes both spontaneous and ACTH induced diureses

If diuresis was the resultant of local vasomotor changes perhaps stimulated by a hormonal mechanism significant changes in effective renal resistance should precede improved filtration *Renal function* studies before and during ACTH therapy and again at the onset of

ALTERATIONS IN RENAL HEMODYNAMICS WITH DIURESIS OF NEPHROTIC EDEMA

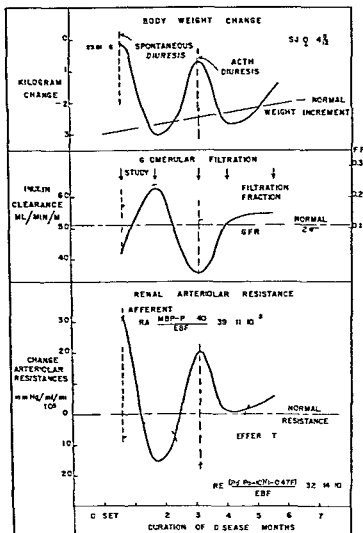


FIG 4

and following diuresis in another patient yielded the observations detailed in Fig 5 Construction of this chart is similar to that of the previous one however the abscissa refers to days rather than months

With therapy early reduction of effective renal resistance possibly resulting from relative dilatation of both afferent and efferent arterioles was associated with a decreased filtration fraction No other hemodynamic changes were noted during the remaining thera

RENAL HEMODYNAMICS DURING ACTH THERAPY AND WITH DIURESIS OF NEPHROTIC EDEMA

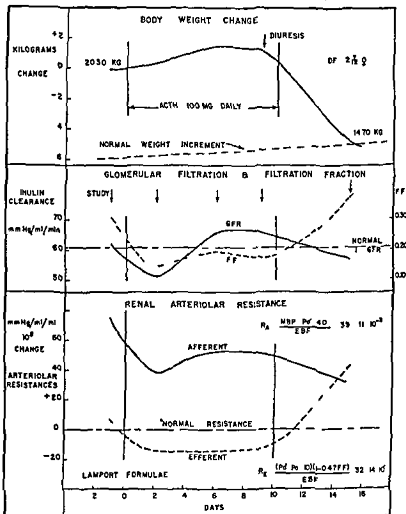


FIG 5

peutic period although diuresis began on the ninth day At that time, increased afferent and decreased efferent resistance were still present An increased filtration fraction was not observed until diuresis was completed This augmentation of filtration fraction appeared to depend upon diminished efferent flow resulting from a relatively striking increase of efferent arteriolar resistance at the end of the period of diuresis

These observations suggest that ACTH diuresis may not be the result of simple hemodynamic adjustments The mechanism of edema formation and ACTH induced diuresis in these children

remains obscure but does not appear to depend upon primary sustained alterations in glomerular dynamics

SUMMARY

ACTH is an extremely useful tool for investigating the pathologic physiology of the nephrotic syndrome in children

Encouraging modifications of the disease have been noted in about two thirds of our treated patients but protracted observation will be necessary to define the ultimate value of the drug in the treatment of this disease

Hypertension hypotonicity and infection are contraindications to or indications for cessation of ACTH therapy in the nephrotic child

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(Editor's Note Since the discussion by Dr John Luetscher Jr is more pertinent to the papers by Drs Metcoff Ingbar and Earle relating to the physiologic aspects of kidney function it is inserted here although part of the discussion is equally pertinent to Dr Farnsworth s presentation in Volume II)

DISCUSSION

DR JOHN LUETSCHER JR The results presented by Dr Farnsworth and Dr Metcoff fill in a number of gaps in our knowledge concerning various actions of ACTH in nephrosis They also emphasize the complexity of the physiological disturbances in nephrosis and the

We are indebted to Drs Nobuyuki Nakasone and William Bergstrom and Mrs Audrey Andrews for valuable assistance in the renal function studies and to Misses M Hasson B Goldberg and M Haling for technical assistance We gratefully acknowledge the splendid cooperation of the nursing and medical house officer staff in the management of the nephrotic children

difficulty in establishing the mechanism of action of the various treatments

Measurements of total formaldehydogenic corticoids and of 17 ketosteroids in the urine tell us that the adrenal cortex responds to ACTH in the normal direction but they offer little help in interpreting the changes in sodium excretion since the various steroids which give these chemical reactions may either increase or decrease the reabsorption of sodium

Dr Q B Deming and I* have measured the sodium retaining action of the corticoid fraction of urine by bioassay in adrenalectomized rats The activity of these extracts of urine from patients with nephrosis and edema has been considerably above the normal level Such high activity is reduced after cortisone therapy when a diuresis follows but remains high when no loss of edema occurs With the assistance of Dr B B Johnson we have followed the changes in sodium retaining activity when ACTH is administered to patients with nephrosis These results can also be correlated with the changes in sodium excretion and edema It seems possible that the adrenal plays a role in the production of edema and that this abnormal activity is reduced after administration of cortisone or ACTH No basis for such abnormal activity of the adrenal has been established and we may equally well be measuring an abnormality of metabolism or excretion The abnormality is associated with an inadequate circulation (heart failure or deficiency of circulating protein) and may be aggravated by an infection

Many other physiological factors are concerned in the proteinuria edema and other manifestations of nephrosis We have been interested to compare some of these with the clinical results of treatment with ACTH or cortisone in 23 patients with nephrosis

In 8 of the 14 cases treated with cortisone in dosage of about 100 mg /day there was complete elimination of edema after treatment although in 3 of these there was some doubt as to whether the cortisone was responsible The remaining 6 patients had no diuresis after cortisone but a subsequent course of treatment with concentrated human serum albumin was followed by diuresis in 5 of the 6 patients in this group

The pattern of a good response to cortisone is shown in Figure 6 This 7 year old boy had an attack of acute glomerulonephritis followed 2 months later by the appearance of the nephrotic syndrome During treatment with cortisone there was temporary accentuation of proteinuria and edema After the end of treatment a diuresis and a reduction in proteinuria ensued with elimination of edema and a

*This investigation was supported by a research grant from the National Heart Institute U.S Public Health Service

return of serum protein and albumin levels nearly to normal At the same time there was a fall in the abnormally high level of sodium retaining activity of the urinary corticoid fraction The serum sodium concentration rose to a normal level Cholesterol concentration fell slowly from 1140 to 485 mg % The abnormally high erythrocyte sedimentation rate was unaffected The clinical remission induced *in this patient* has lasted for 8 months Other cases have not been so fortunate A remission may be interrupted by an acute infection or there may be a gradual return of edema for no apparent reason

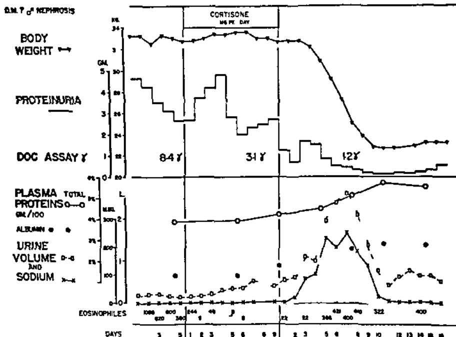


FIG 6 *Remission of Nephrotic Syndrome after Cortisone* DOC assay indicates the equivalent in micrograms of desoxy corticosterone of the sodium retaining activity of the urinary corticoid fraction

In 7 of the 10 cases treated with ACTH in dosage of 50 mg /day edema has been eliminated In one patient the response did not follow the usual pattern and the diuresis was ineffectual Two patients lost no edema during or after ACTH treatment in both of these patients signs of an acute infection became apparent when ACTH was withdrawn *Over half of the patients treated with ACTH* have shown a marked reduction in proteinuria during or after treatment

A number of similarities in the effects of ACTH and cortisone have been observed (Figure 7). Among these are the increases in creatinine and inulin clearance during treatment, the tendency to retention of sodium and water during the initial days of treatment and to release on later days, the release of sodium and water on withdrawal of treatment, and the diminution in proteinuria which commonly occurs at this time. Neither drug regularly affects the abnormal erythrocyte sedimentation rate. The serum cholesterol may

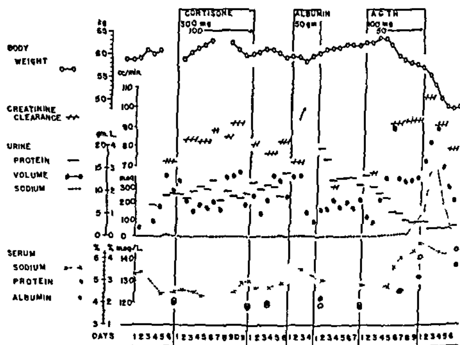


FIG 7 *Remission of Nephrosis after ACTH following Failure of Cortisone and Albumin to Induce Diuresis*

fall after either drug, especially if a remission occurs, but the level usually remains well above normal.

The observed differences between the actions of cortisone and those of ACTH must be interpreted cautiously because of inevitable differences in small groups of patients. In general ACTH is followed by an earlier and more impressive elimination of water and sometimes of sodium during the later days of treatment; perhaps for this reason a rise in serum potassium level has been less often observed than during cortisone administration. The diuresis at the end of ACTH administration is generally more abrupt and profuse than after cortisone. Proteinuria may fall to very low levels during ACTH

treatment but may recur very rapidly after the end of therapy (Figure 8)

In considering the mechanism of diuresis during ACTH administration it may be of interest that the sodium retaining activity of the urinary corticoid fraction may fall during this phase (Figure 8) Since the stage is set for diuresis by the increased glomerular filtration rate and by the reduced loss of protein the rejection of sodium

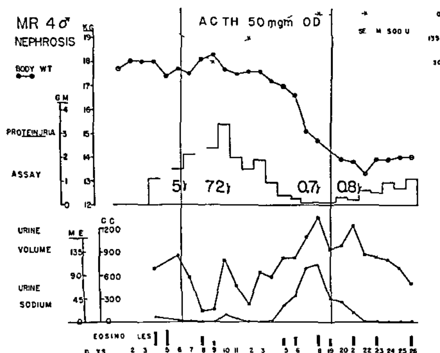


FIG 8 *Diuresis Reduction of Proteinuria and Fall in Assay of Sodium Retaining Corticoid during ACTH Administration*

by the renal tubules may provide the last necessary element for diuresis

There has been so much variation in the duration of remissions after treatment with either ACTH or cortisone that no comparison of the two agents on this basis is possible Much longer observation will be necessary before an estimate of the effects of treatment on the renal lesion can be made On the basis of improvement in abnormalities of renal function and of urinary sediment it would appear that a few patients with active and progressive renal lesions have been substantially helped

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The Effect of Sodium and Potassium on the Metabolic and Physiologic Responses to ACTH*†

William Ransohoff, Albert A Brust ‡ Morton F Reiser, I Arthur Mirsky and Eugene B Ferris

CINCINNATI GENERAL HOSPITAL UNIVERSITY OF CINCINNATI COLLEGE OF MEDICINE AND THE MAY INSTITUTE FOR MEDICAL RESEARCH OF THE JEWISH HOSPITAL CINCINNATI

In an effort to throw further light on the interrelationship between sodium metabolism and adrenal cortical activity a study was designed to evaluate the effect of sodium balance on the metabolic and physiologic responses to ACTH In the course of the study it became apparent that some of the changes noted during variations in sodium intake were associated with simultaneous changes in potassium balance so that attention was also directed to potassium balance

MATERIAL AND METHODS

Three patients were studied (1) R H a 42 year old colored woman with uncomplicated essential hypertension (2) G L a 39 year old colored woman with essential hypertension complicated by impaired renal function and congestive heart failure and (3) M T a 45 year old white woman with normal blood pressure and rheumatoid arthritis of 18 months duration

The patients were observed in each of five periods

Period A High normal sodium intake (4 2–5 0 grams sodium/day) (high sodium period)

Period B Restricted sodium intake (0 2–0 3 grams sodium/day) (low sodium period)

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* This work was supported in part by grants from the American Foundation for High Blood Pressure and the U S Public Health Service

† We are indebted to Miss Gladys Perisutti for her technical assistance and to Dr Alvin I Shapiro and Dr William N Chambers for their help

‡ Research Fellow American Heart Association

Period C Restricted sodium intake plus ACTH (100 mg/day) (ACTH–low sodium period)
Period D High normal sodium intake plus ACTH (ACTH–high sodium period)
Period E High normal sodium intake (high sodium period)

The sequence of periods and duration varied with each patient and are summarized in Table I

Table I

Duration and Sequence of Periods

Patient	*(1) R H*	*(2) G L*	*(3) M T*
Clinical diagnosis	*Uncomplicated hypertension*	*Hypertension Heart failure Renal damage*	*Rheumatoid arthritis Normal BP*
	(A) high sodium 11 days	(A) high sodium 30 days	(A) high sodium 12 days
	(B) low sodium 17 days	(B) low sodium 30 days	(D) ACTH–high sodium 28 days
	(C) ACTH–low sodium 9 days	(C) ACTH–low sodium 27 days	(C) ACTH–low sodium 20 days
	(D) ACTH–high sodium 6 days	(D) ACTH–high sodium 7 days	(D) ACTH–high sodium 1 day
	(E) high sodium 12 days	(E) high sodium 22 days	(E) high sodium 35 days
Total days ACTH	15	34	49
Total days study	55	116	96

It should be noted that in the first two patients the period of ACTH–high sodium followed the period of ACTH–low sodium In the third patient the sequence of these periods was reversed

The patients were hospitalized on the medical wards of the Cincinnati General Hospital At the time of admission each patient was placed on a 0 2 gram sodium diet the sodium intake was then controlled by giving either lactose or sodium chloride tablets depending upon the period In like fashion hypos of physiologic saline were interchanged with ACTH depending upon the period The potassium intake was within normal limits and was not supplemented except where indicated

Daily measurements were made of the weight pulse blood pressure dietary and fluid intake urine volume and its sodium potassium and sugar content At one to five day intervals the following studies were made serum sodium and potassium urinary 17 ketosteroid excretion complete blood count with hematocrit eosinophil count ballistocardiogram electrocardiogram and the response of the blood pressure to the rapid intravenous administration of 400 mg of tetraethylammonium chloride (TEAC) an autonomic blocking agent and in the third patient electroencephalograms were taken At least once in each period of study a glucose tolerance test was done In addition in the first patient urine uric acid and creatinine determinations blood volume and insulin tolerance tests were done In the second patient, periodic venous pressure and circulation time studies were made

The hematologic studies were done in the usual fashion The eosinophil counts were done according to the method suggested by Thorn[1] Serum sodium and potassium determinations were made on the flame photometer[2] The ballistocardiographic studies were done with the assistance of Dr John Braunstein on an instrument of his design[3] Twenty four hour specimens of urine were collected with a few crystals of thymol as preservative and determinations were run on aliquots for sodium and potassium using the flame photometer[2] sugar according to Nelson s[4] adaptation of the method of Somogyi[5] and 17 ketosteroids according to the method of McCullaugh[6]

RESULTS

In Case 1 control values were obtained in the initial period of high sodium intake (Fig 1) Sodium restriction alone effected a two pound weight loss and a slightly negative sodium balance (A true sodium balance was not measured however for convenience the calculated sodium intake minus the measured urinary sodium excretion is hereafter referred to as sodium balance) There were no significant changes in urinary excretion of 17 ketosteroids total leukocyte or eosinophil count and the urine remained sugar free The relationship between the sodium intake and the cardiovascular system is discussed elsewhere[7]

On the eighteenth day of sodium restriction ACTH was started (25 mg every six hours) and sodium restriction continued There was a moderate rise in urinary 17 ketosteroid excretion a prompt but transient glycosuria an increase in the total leukocyte count and the eosinophils disappeared from the peripheral blood

After nine days of ACTH-low sodium the sodium intake was increased from 0 2 to 4 2 grams per day and ACTH continued for six

days There was a prompt weight gain indicating water retention increased sodium retention a further rise in urinary 17 ketosteroid excretion and again there was a prompt and greater but again transient glycosuria The total leukocyte count rose to 20 000 per cubic millimeter and the eosinophil count remained depressed

When ACTH was discontinued all the values fell promptly to or below the initial control levels There was a prompt and marked diuresis effecting a 14 pound weight loss in six days In this six day period the amount of sodium excreted in the urine far exceeded the

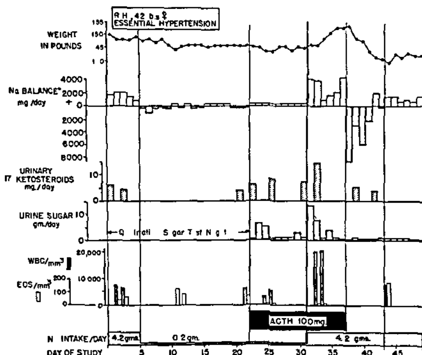


FIG 1 The effect of sodium intake on the response to ACTH Case I

sodium intake and certain of the cardiovascular responses[7] were comparable to those obtained in the period of sodium restriction The serum sodium remained within normal limits throughout the study

The second patient was followed in similar fashion and the responses were comparable to those just described except that this patient retained excess water regardless of the sodium intake and at no time developed any glycosuria

In the third patient maximum responses to ACTH were obtained in a 28 day period of ACTH–high sodium (Fig 2) In order to see if the effect of a high sodium intake on the responses to ACTH

could be reversed by the restriction of sodium the sodium intake was then reduced to 0 2 gram per day and ACTH continued for 21 more days

For the first 12 days of the ACTH–low sodium period the urinary 17 ketosteroid excretion glycosuria and leukocytosis continued the upward progression begun in the preceding period of ACTH–high sodium however there was a slight weight loss and the sodium balance was slightly negative during this time On the thirteenth day

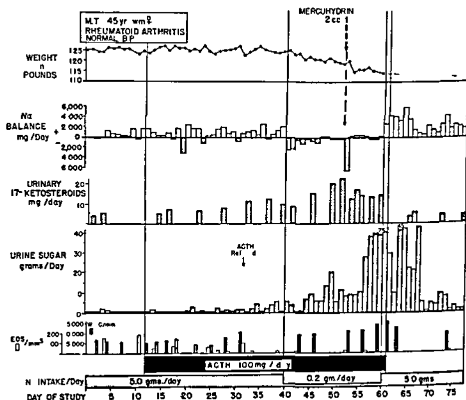


FIG 2 The effect of sodium intake on the response to ACTH Case 3

of the ACTH–low sodium period in an effort to expedite sodium depletion mercuhydrin was given in the ensuing 24 hours the urinary volume increased markedly there was a corresponding five pound weight loss and a marked sodium loss (Fig 2)

Following this accelerated desalting the urinary 17 ketosteroid excretion fell from 22 5 to 12 5 mg per day at which level it remained for the duration of the ACTH–low sodium period There was a transient reduction in glycosuria which may be related to the desalting process since the effects of sodium restriction do not become apparent immediately when the sodium intake is reduced In any case the glycosuria decreased only temporarily and sodium restric

tion did not prevent a subsequent rise Changes in fasting blood sugar were never commensurate with the glycosuria In this patient then the 17 ketosteroid effect of ACTH was inhibited by sodium restriction Perhaps also the glycosuric effect was temporarily inhibited The serum sodium was at no time below 130 milliequivalents per liter

In every case when ACTH was started there was an immediate feeling of well being however within 10 to 11 days all the patients complained of weakness and nervousness Ileus was noted in two cases In Case 1, post ACTH the patient became more anxious and for several days had transient paranoid ideation without gross evi

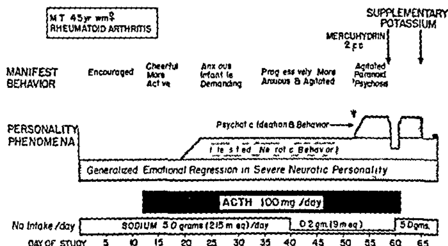


FIG 3 Changes in manifest behavior and personality phenomena during the administration of ACTH Case 3

dence of major psychosis In Case 2 after an initial improvement the patient became progressively depressed and seven days post ACTH developed a non specific psychotic state which clinically resembled an organic psychosis and was characterized by severe regression withdrawal infantile behavior and profound loss of cortical integrative and intellectual function

Because of these observations an independent psychiatric observer Dr Norman Chivers had frequent interviews with the third patient and in addition serial electroencephalograms were taken and interpreted by Dr J Park Biehl

This patient was an anxious easily disturbed woman with many neurotic traits and has been characterized as a severe neurotic personality (Fig 3) The psychiatric changes noted even before ACTH administration consisted primarily of a generalized emotional regression consistent with the experimental situation and the patient s

personality pattern In addition on the forty third day of ACTH there was superimposed a gross paranoid psychosis

Figure 3 depicts changes in personality phenomena and manifest behavior At first the patient was encouraged and more active how ever she gradually became more anxious infantile and demanding This pattern has been characterized as intensified neurotic be havior and is superimposed on the baseline of the generalized emotional regression in a severe neurotic personality Her anxiety progressed and finally could not be relieved by constant reassurance

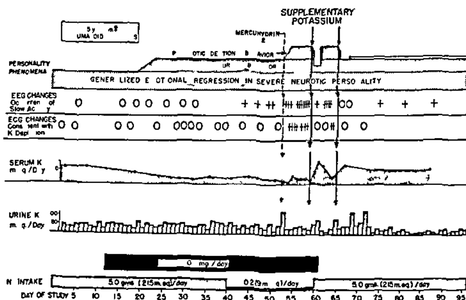


FIG 4 Changes in personality phenomena during ACTH administration Case 3 correlated with physiologic changes related to potassium depletion

she became agitated and on the forty third day of ACTH she became frankly psychotic

In Figure 4 the changes in personality phenomena are correlated with physiologic changes consistent with potassium depletion namely changes in serum potassium the electroencephalogram and the electrocardiogram The electroencephalographic changes were in the form of bursts of slow activity the occurrence of which we have graded from one to four plus Simultaneous electrocardiographic changes consistent with potassium depletion we have graded as one or two plus The urinary excretion of potassium is also charted Slow activity appeared in the electroencephalogram on the thirty second day of ACTH at which time the patient was agitated and anxious On the forty first day mercuhydrin was given for desalting pur

poses there was a simultaneous increase in urine potassium excretion and a further fall in serum potassium In an electroencephalogram taken in the course of the diuresis bursts of slow activity occurred more frequently the next day the T waves of the electrocardiogram became iso electric charted as a two plus change and within 36 hours the patient was frankly psychotic Four days later she was still psychotic and electroencephalographic and electrocardiographic changes were marked Six grams of potassium chloride were given orally at this time Within 12 hours the patient was no longer psychotic the electroencephalogram showed only a rare burst of slow activity and the electrocardiogram was normal However the next

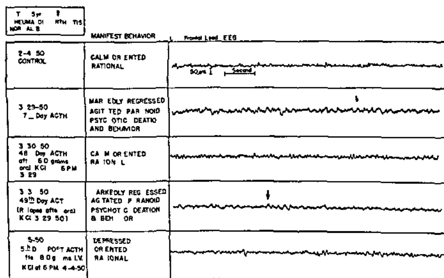


FIG 5 Representative electroencephalographic changes in the various periods of potassium imbalance Case 3

day in the course of a glucose tolerance test the patient again became psychotic the electroencephalogram abnormal There was no change when ACTH was discontinued and in addition electrocardiographic abnormalities reappeared Four days post ACTH supplementary potassium (400 cc of 2% potassium chloride intravenously over a three hour period) was again given and the disappearance of the psychosis and the improvement in electroencephalogram and electro cardiogram were as dramatic as previously (The pH of the blood was not determined during this study the CO combining power was within normal limits throughout the investigation)

In Figure 5 are shown representative samples of the electroencephalograms taken during the various periods of potassium imbalance all tracings are the left frontal lead In the control period the

patient was calm and oriented the electroencephalogram normal On the forty seventh day of ACTH she was psychotic and bursts of slow activity are present The next day after oral potassium the psychosis disappeared and the electroencephalogram was essentially normal There was then a relapse in both clinical behavior and the

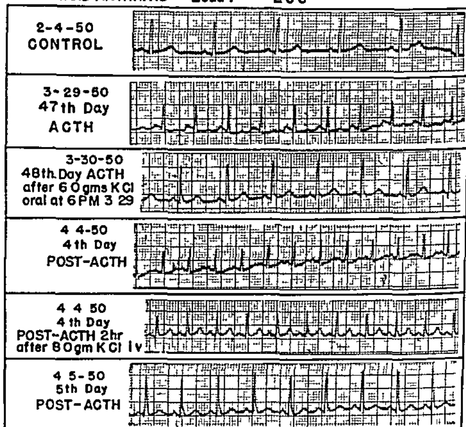


FIG 6 Representative electrocardiographic changes in the various periods of potassium imbalance Case 3

electroencephalogram and both were again corrected by the administration of potassium

Thus the data suggest that there is a definite correlation between the psychotic state electroencephalographic changes and potassium depletion

In Figure 6 are shown representative samples of the electrocardiograms taken during the various periods of potassium imbalance The leads are all Lead 1 Most apparent are the changes of the

T waves upright in the control depressed at the time of potassium depletion and their reappearance disappearance and reappearance again correlated respectively with the administration depletion and again the administration of potassium ST segment shifts can also be seen but definite prolongation of the Q T interval was not observed

SUMMARY AND DISCUSSION

Data have been presented in an effort to demonstrate the effect of sodium intake on the metabolic and physiologic responses to ACTH In Case 1 increasing the sodium intake following a period of ACTH–low sodium was accompanied by an increase in the urinary excretion of 17 ketosteroids and sugar and the white blood count

Conversely in Case 3 restriction of sodium followed a period of ACTH–high sodium inhibited the urinary excretion of 17 ketosteroids and perhaps temporarily inhibited the glycosuria The white blood count in Case 3 was not materially altered Changes in the humoral component of the blood pressure and other vascular responses to ACTH also appear to be greater when the sodium intake is not restricted These data suggest that the sodium ion may well be actively involved in the response to ACTH and/or the activity of the adrenal cortex and that variations in the sodium intake may in part explain some of the variations in response to ACTH that have been noted by others

Certain of the toxic side effects of ACTH administration have been observed namely excessive nervousness weakness electrocardiographic changes and ileus These findings are consistent with a state of potassium depletion In addition the third patient became frankly psychotic simultaneous electroencephalographic changes comparable to those previously reported by Hoefer and Glaser[8] and electrocardiographic changes consistent with potassium depletion were obtained and the psychosis disappeared and the electroencephalogram and electrocardiogram returned to normal following the administration of potassium

The severity of the effects of potassium depletion depended upon the duration of ACTH administration for although serum potassium levels tended to fall during the administration of ACTH day to day variations did not clearly correlate with the clinical severity of deficient symptoms This was particularly apparent when potassium chloride was administered to the third patient The psychosis temporarily disappeared and the electroencephalogram and electrocardiogram improved The psychosis and the electroencephalogram then relapsed before the electrocardiogram again became abnormal and the serum potassium did not again become abnormally low until

three days after clinical evidence of potassium deficiency had again manifested itself

These observations might be interpreted as indicating that the clinical evidence of potassium depletion is dependent upon that ion s intracellular concentration and that the serum level does not clearly reflect the presence or absence of potassium depletion

CONCLUSIONS

The data presented suggest

(1) that variations in sodium intake influence certain metabolic and physiologic responses to ACTH and in particular the urinary excretion of 17 ketosteroids and perhaps the urinary excretion of sugar

(2) that certain of the toxic side effects of ACTH administra tion may be actually due to potassium depletion All of the changes in behavior associated with the administration of ACTH may not be attributed to potassium depletion alone However the psychotic component correlated with changes in the electroencephalogram both of which were corrected by the administration of potassium seems to reflect potassium depletion and can best be defined as a toxic psychosis or delirium

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DISCUSSION

DR GILBERT GLASER (College of Physicians and Surgeons Columbia University The Neurologic Institute New York City) This demonstration of the effect of potassium depletion represents a significant advance in our understanding of the mechanism of production of electroencephalographic and mental changes appearing in the hyperadrenal state

However the effect of potassium depletion on central nervous system activity indicates only one phase of a very complex process For example we have observed one patient with chronic thyroiditis and myxedema who developed a severe psychotic reaction with delirioid depressive and paranoid features towards the end of a course of ACTH therapy becoming exaggerated within 24 hours after the medication was discontinued This was associated with the appearance of slow activity in the EEG Both the EEG changes and the psychosis cleared within a very short time on the administration of thyroid medication

There seem to be two major groups of severe psychotic reactions induced by ACTH or cortisone One in which signs of an organic mental syndrome such as a toxic delirium are present and one without these manifestations

In both affective and schizophreniform phenomena occur Manic psychoses seem to be somewhat special they develop in transition from euphoria and elation occurring as chronic or painful disabling symptoms are relieved and usually appear without organic features being present

The pattern or content of these reactions is in most cases related to the previous personality structure of the patient However only in some but not all cases have we been able to demonstrate abnormal pre psychotic personality patterns

Also it should be noted that severe psychoses can occur in the absence of EEG changes and vice versa

Serum potassium levels certainly are inconsistent and not correlated nor have we been able to correlate electrocardiographic

changes which have been suspected to have been due to potassium deficiency In other words we have seen patients with marked EEG changes and no changes in the electrocardiogram

In relation to these phenomena we have studied the responses of six schizophrenic patients to large doses of ACTH 100 milligrams for 30 days in four patients and 200 milligrams a day for 10 days in two patients Five patients showed no change whatsoever in their psychotic manifestation One patient a 23 year old girl with a mixed schizophrenia predominantly paranoid developed exaggerated catatonic features within 24 hours after the ACTH was discontinued

Also we have noted no specific euphorizing effect of the drug even in patients with depressive tendencies

We are now seeing a relatively large number of these induced psychotic states The exact incidence is not known We feel that in many cases these reactions are unpredictable and can occur despite presumably adequate psychological screening

I would like to emphasize one other significant probable effect of potassium depletion which was alluded to by Dr Ransohoff and that is the appearance of marked muscular asthenia This tends to occur particularly in patients with muscular and neuromuscular disorders such as amyotrophic lateral sclerosis muscular dystrophies myasthenia gravis and dermatomyositis Again there is no correlation with the serum potassium level In some of these cases however the response to potassium administration even with large dosages is minimal and it is quite possible that a more complex mechanism is involved

DR JOHN R MOTE At the time of the first ACTH Conference I believe that we were all impressed with the incidence of psychoses in patients under therapy with ACTH It should be recalled however that practically all of the early studies with ACTH were on a rather standard dosage of 100 mg per day regardless of the disease syndrome or the purpose of the experiment Likewise it should be recalled that a high proportion of the patients studied were on either complete or semi complete metabolic balanced studies with a fixed intake of salt and potassium

In view of the evidences presented at the First Conference indicating that there are marked shifts in electrolytes during these high dosage schedules post conference trends became rapidly evident

First many investigators began using lower doses of ACTH in many disease syndromes and second most investigators undertook some restrictions of sodium chloride intake and began administering supplements of potassium salts

The thing that has struck me over all is the much lower incidence

of psychoses reported to us since these two trends have developed which lends some credence to Dr Ransohoff's observations I am primarily *impressed at this stage with the uncommoness of the development of real psychosis in patients on adequate therapeutic regimens with attention paid to the electrolyte trends*

DR ROBERT A CLEGHORN I believe that this is a very striking communication which we have just heard I think time will show that the findings of the Cincinnati group have a key place in the understanding of psychiatric and physiological changes associated with adrenal cortical secretion My interest in this field concerns psychiatric patients particularly and some report of our work may be appropriate at this juncture

A Effect of ACTH on Depression

Eight cases of depression have been treated with ACTH in a study which was made in conjunction with Drs Cameron and Saffran

Table II gives some of the data on these patients They received

Table II

Case #	*Sex*	*Age*	*Duration*	*Total Dose (mg equiv)*	*Weight gain (lbs)*
1	m	42	6⅓	662	8
2	f	49	7	487	10
3	m	33	7	500	4
4	f	45	8	625	5
5	f	45	9⅓	737	24
6	f	26	12	862	11
7	f	48	16⅓	1350	7 (convulsion
8	f	38	20	1500	15

ACTH every four hours for a number of days in a dose of approximately 100 milligrams daily and thereafter in somewhat lesser amounts A variety of psychological and psychophysiological tests were carried out The tests yielded relatively little information In only one case were slow wave changes seen in the electroencephalogram Blood and urine studies indicated marked activation of the adrenal cortex Clinical psychiatric changes were slight inconstant and temporary In all patients ECT would have been the usual treatment on admission and it was given to seven of the eight patients with satisfactory results subsequent to the course of ACTH Three patients in whom treatment was continued for twelve to twenty two days showed no further clinical benefit

One case developed a serious complication spontaneous convulsions This woman aged 48 with agitated depression at the menopause was receiving ACTH in four hourly doses On the seventeenth day of treatment (total dose 1350 mg) she complained of intense headache and a few hours later had a generalized convulsion followed by ten severe grand mal seizures in the next fifteen hours Most of the time between seizures she was comatose Neurological examination showed some abnormal reflexes CSF was under increased pressure but otherwise normal Thirty hours after the first convulsion she responded somewhat and complained of headache Neurological examination became normal after a few days The patient had *no previous history of seizures* and no recognized cause of convulsions could be established in this case independent of the ACTH

Post-convulsive EEG records showed some disorganization which returned to normal within five months To date there has been no further convulsion and no neurological evidence of a cerebral lesion This patient was not given ECT As a result of discussions with Drs Ransohoff and Glaser I suspect that these convulsions may have had their basis in potassium changes

B Acute Depression Following Cortisone

I would like to underscore what Dr Glaser has said about the absence of pre psychotic personality in some individuals developing mental changes with hormone treatment as witnessed by the following experience

A laboratory worker with a chronic skin condition believed by several consulting dermatologists to be chronic lupus erythematosus was given 600 mg of cortisone in three doses over a period of one week *She then experienced a rather abrupt onset of extreme pessimism* psychomotor retardation insomnia apprehension suicidal thoughts and ruminations about past tragedies This progressed for a few days and then regressed while the patient was given dexedrine sedative and reassurance Recovery seemed complete in about one week

C Eosinophils in Manic Depressive Patients

Some observations on daily eosinophil counts in manic depressive patients proved to be of interest The eosinophil response to ACTH was normal in eight of ten manic-depressive patients observed Basal eosinophil counts increased steadily during a course of ECT A sudden decrease preceded clinical evidence of relapse into depression

DR IRVINE MCQUARRIE In one of the experiments of nature we see something of sort noted in Dr Cleghorn s last case In 1937 Johnson Ziegler and I described the first case of Cushing s syndrome in which there was observed a marked disturbance of the electrolytes almost diametrically opposite that of Addison s disease except that the chloride was low in this case as in Addison s disease

The serum potassium was in the neighborhood of 2 0 to 2 5 milli equivalents per liter instead of the usual 4 5 to 5 5

The plasma CO content was in the neighborhood of 112 to 116 volumes per cent or around 40 to 45 milli equivalents per liter instead of 25 to 28

The serum chlorides were between 80 and 88 instead of the normal of 100 to 104 milli equivalents per liter

The sodium in that patient was equivalent to the total base in the average normal individual about 155 milli equivalents

This 40 year-old patient with Cushing s syndrome had been a most amiable and well adjusted person before developing her illness All of her relatives were greatly embarrassed by her amazing change in behavior and personality They said Why she acts at times as if she is crazy She was greatly depressed or disturbed had poor memory and did very odd things Her sister bemoaned the fact that she had no modesty whatsoever

We gave her fairly large amounts of potassium chloride instead of extra sodium chloride that one would give for Addison s The patient s personality change was one of the most striking features of the response when her serum electrolyte pattern was thereby returned to normal temporarily

DR ALBERT DORFMAN (Bobs Roberts Memorial Hospital University of Chicago School of Medicine Chicago) In the course of our studies of the effects of ACTH on connective tissue we have had three patients develop status epilepticus while receiving ACTH The patients had dermatomyositis rheumatic fever and periarteritis nodosa All three patients showed elevated spinal fluid proteins Two of the patients had complete recovery while one has evidence of severe neurological damage eight months later

DR SHELDON MARGEN Another observation of some interest is the relation between sodium intake and hyperglycemia in some individuals receiving ACTH Some patients who have excessive fluid retention and also hyperglycemia will have disappearance of the hyperglycemia when the edema is controlled by restriction of sodium intake This may be referable in part at least to hepatic edema with consequent impairment of glycogen storage

DR WILLIAM RANSOHOFF In comment on Dr Glaser s discussion we certainly agree that all of the emotional changes noted during the administration of ACTH cannot be attributed to potassium depletion alone We further feel that possibly the premorbid personality merely dictates the nature of the emotional reaction which the patient may have

We are in complete agreement with the comment that the serum potassium is not an adequate measure of potassium depletion We do feel that electrocardiographic changes may be used as an indication of a severe deficiency of this ion—and in the data presented electrocardiographic changes consistent with potassium depletion were demonstrated Further it should be noted that the electroencephalographic changes appeared earlier in the course of ACTH administration—and earlier in the course of potassium depletion—than did the electrocardiographic changes and that the electroencephalographic changes were corrected a little less rapidly by the administration of potassium This suggests that in this case at least the electroencephalogram was a more sensitive measure of potassium depletion than the electrocardiogram

In reference to Dr Cleghorn s comments we have observed emotional changes in three non psychotic patients who were given ACTH The frequency of psychiatric changes reported during the administration of ACTH certainly warrants further investigation of the effect of ACTH on psychotic patients and Dr Cleghorn s observations are noteworthy

Dr McQuarrie s comments about the patient with Cushing s Syndrome and hypokalemia are most fascinating and certainly the observations that he presented are in keeping with ours

Dr Margen s observations on the effect of sodium on the response to ACTH seem to be somewhat but not completely at odds with our observations We agree that the hyperglycemia caused by ACTH was not significantly altered by varying the sodium intake however the increase in glycosuria on increasing the sodium intake was demonstrated particularly in the first case presented This we believe was primarily a renal glycosuria and does not indicate any overall change in carbohydrate metabolism accomplished by the giving or restricting of sodium

In regard to the effect of ACTH on carbohydrate metabolism it is worthy of comment that in the third case presented the case with psychiatric and electroencephalographic changes and marked glycosuria the glucose tolerance curve became markedly abnormal Further acetone appeared in the urine in this patient on the forty eighth day of ACTH and temporarily exceeded 1 0 gram per day

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Vascular Responses to ACTH and Alterations in Sodium Intake*

Albert A Brust,† William Ransohoff, Morton F Reiser and Eugene B Ferris

CINCINNATI GENERAL HOSPITAL UNIVERSITY OF CINCINNATI COLLEGE OF MEDICINE AND THE MAY INSTITUTE FOR MEDICAL RESEARCH OF THE JEWISH HOSPITAL, CINCINNATI

Thus far there has been no clarification of the vascular changes which have been reported as a consequence of ACTH administration Hypertension encephalopathy cerebral accident and cardiac failure have been observed[1] Presumably these complications have accompanied the water and electrolyte changes induced by the drug however convincing evidence that sodium retention or other specific metabolic change is primarily responsible for these occurrences has been lacking

In a previous study Stead Reiser Rapoport and Ferris[2] demonstrated that the feeding or restriction of sodium in essential hypertensives with adequate renal function consistently produces a respective increase or decrease in non neurogenic or humoral vascular tone as measured by the blood pressure response to tetraethylammonium chloride (TEAC) an autonomic ganglionic blocking agent These changes occurred without significant alteration of serum sodium levels However when patients with renal insufficiency were subjected to alternate periods of normal and restricted sodium intake the phenomenon was not observed although the serum sodium often fell to very low levels Figures 1 and 2 illustrate these changes in a representative patient of each group These observations suggested the activity of a renal mechanism and perhaps the involvement of adrenocortical factors in producing these alterations in the components of vascular tone The current study was designed in an effort to further clarify this phenomenon and also to investigate the reported pressor and vascular effects of ACTH

* (This work was supported in part by grants from the American Foundation for High Blood Pressure and the U S Public Health Service)

† This work was done during the tenure of a Research Fellowship of the American Heart Association

EFFECT OF SALT BALANCE ON BLOOD PRESSURE AND TEAC FLOOR

FIG 1 This is a record of the blood pressure 1nd TEAC floor changes produced by alternate periods of salting and desalting These data are representative of the results obtained in a group of hypertensives with adequate renal function The thin vertical line indicates the control blood pressure and the wider shaded line the TEAC floor The encircled figures indicate serum sodium values in milliequivalents per liter

EFFECT OF SALT BALANCE ON BLOOD PRESSURE AND TEAC FLOOR

FIG 2 This record is representative of the results of salting and desalting in hypertensive patients with renal insufficiency The legend is the same as for Figure 1

MATERIAL AND METHODS

The action of ACTH in doses of 100 mg per day was studied in the following patient types

1 Uncomplicated essential hypertension
2 Essential hypertension moderate renal impairment and chronic congestive failure
3 Normotension with rheumatoid arthritis
4 Normotension two weeks following postpartum recovery from pre eclampsia

The first three patients were studied during periods of high normal (4 2–5 Gms /day) and restricted (0 2 Gms /day) sodium intake Technique of metabolic studies was as described by Ransohoff et al [3] in a concurrent investigation The fourth patient received normal sodium intake during the period of observation and metabolic studies were not performed in this patient

Tetraethylammonium chloride* was administered daily or on alternate days in doses of 4 cubic centimeters (400 mg) injected intravenously The technique of TEAC administration and the method of determining control blood pressure and TEAC floor have been described in detail elsewhere [4] The sphygmomanometer was used for blood pressure determinations and all measurements were made with the patients supine For purposes of presentation the blood pressures and TEAC floors in certain of the figures represent the mean of a week s observations and include from three to six tests

The hypertensive patients were begun on ACTH after prolonged sodium restriction had resulted in slight fall in the blood pressure and more pronounced lowering of the TEAC floor In the normotensive patients ACTH was started during normal sodium intake

RESULTS

In all patients the TEAC floor rose above control levels within 24 hours after ACTH was begun and remained elevated throughout Following discontinuance of the drug the TEAC floor fell to low levels concomitant with sodium diuresis and other evidence of reduced adrenal activity Rise in the TEAC floor consistently was demonstrated to precede the increase in urinary excretion of 17 keto steroids and to precede or parallel depression of circulating eosinophiles Although marked lowering of TEAC floors accompanied sodium diuresis when ACTH was stopped weight charts did not in

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* Etamon chloride furnished by Parke Davis and Company through the courtesy of Dr E C Vonder Heide

dicate water retention when TEAC floors rose in response to ACTH administration

In general blood pressure alterations paralleled the TEAC floor changes but in the hypertensive patients these were of minor magnitude On ACTH the normotensive patients promptly developed moderate hypertension which persisted until the drug was stopped In addition to the blood pressure and TEAC floor changes metabolic studies indicated that shifting of the sodium balance appears to exert a specific though quantitatively slight influence on the adrenal stimulating effect of ACTH This was especially evident during the change from restricted to normal sodium and following withdrawal of ACTH

The results in a patient with uncomplicated essential hypertension are illustrated in Figure 3 After a control period on normal

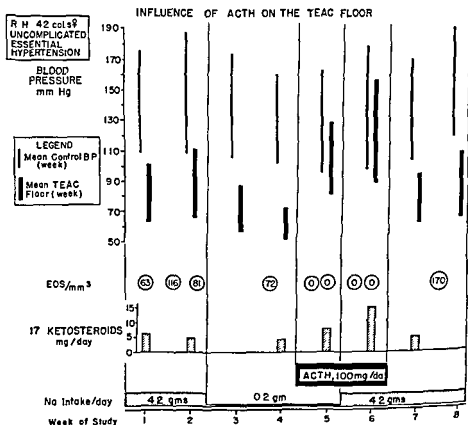


FIG 3 The week of study and sodium intake are indicated at the bottom of the figure The thin vertical lines indicate the control blood pressure and the wider shaded lines the TEAC floor Both values here represent the mean of a week s observations and include from three to six tests with TEAC

sodium intake restriction produced a slight fall in control blood pressure and more pronounced fall in "TEAC floor." When ACTH was begun the "TEAC floor" rose promptly concomitant with a slight rise in urinary 17 ketosteroids. When normal sodium intake was restored both of these values rose even higher. When ACTH was discontinued the "TEAC floor" fell promptly to control levels.

Similar changes are depicted in Figure 4 in the studies of a pa-

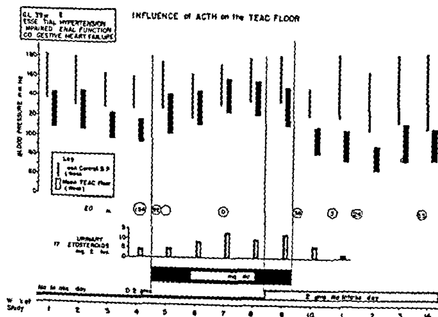


FIG 4 The week of study and sodium intake are indicated at the bottom of the figure. The thin vertical lines indicate the control blood pressure and the wider shaded lines the "TEAC floor." Both values here represent the mean of a week's observations and include from three to six tests with TEAC.

tient with essential hypertension, moderate impairment of renal function and persistent congestive failure at bedrest. ACTH had to be discontinued in this patient after 33 days because of progressive azotemia. Transient but definite improvement in the heart failure was indicated by decrease in dyspnea and decreased circulation time and venous pressure during the first 10 days of ACTH. Thereafter failure recurred to the same clinical extent as before ACTH. When normal sodium intake was added in the ninth week of study the failure worsened but because of the many variables it is difficult to be certain that the sodium alone was responsible.

Figure 5 illustrates the day to day studies in a normotensive pa-

tient with rheumatoid arthritis A prompt rise in control blood pressure and even greater rise in TEAC floor occurred when ACTH was begun From the 12th to the 25th day of study the TEAC floor continued to rise while eosinophiles were only moderately depressed It was then learned that the ACTH being used was only partially potent and when a more potent preparation was substituted eosinophiles disappeared and the blood pressure and TEAC floor rose higher At times the response of the blood pressure to TEAC became entirely pressor Further evidence for the sensitivity of the TEAC mechanism was noted on the 32nd day of study when the refusal of

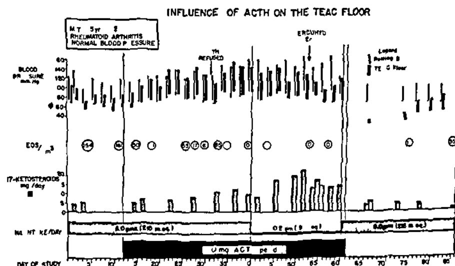


Fig 5 This figure illustrates the day to day studies in a normotensive patient The legend is the same as for previous figures

two doses of ACTH resulted in a fall in TEAC floor and a rise in eosinophiles to 85 From the 40th to the 60th day sodium was restricted during ACTH administration and TEAC floors fell progressively although the control blood pressure remained elevated Toward the end of this period the 17 ketosteroids likewise fell moderately * When ACTH was discontinued the control blood pressure promptly reverted to normal and TEAC floors fell to very low levels

Especially significant portions of the day to day blood pressure and TEAC floor changes in two of the patients are illustrated in Figure 6 In hypertensive patient R H are depicted the prompt step like rise in TEAC floor in response to the administration of ACTH

* Mercuhydrin (2 cc) was given intramuscularly on the 53rd day of study to hasten sodium diuresis

and the likewise immediate fall in floor which occurred concomitant with sodium diuresis when the drug was stopped The data on normotensive patient M T shows the rise of TEAC floor with ACTH and also the progressive fall in the floor value when sodium was restricted during ACTH although hypertension was sustained

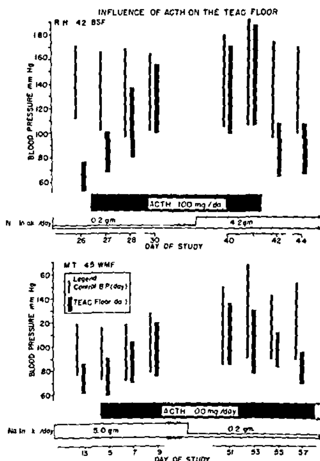


FIG 6 Significant portions of the day to day blood pressure and TEAC floor changes as observed in hypertensive patient R H and normotensive patient M T

In Figure 7 are shown the day to day studies in a normotensive patient two weeks following postpartum recovery from severe pre eclampsia * Maintained on normal sodium intake throughout the study she developed hypertension with ACTH which persisted for several days although only 200 mg of the drug were given The re

* We are indebted to Dr Nicholas Assal Department of Obstetrics for assistance with the studies on this patient

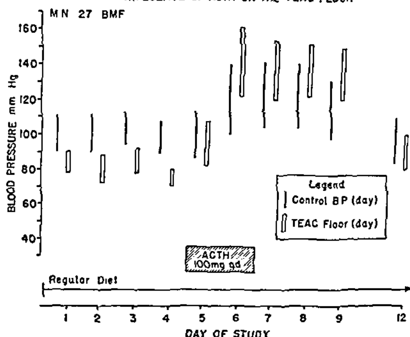


FIG 7 Here illustrated are the day to day studies of normotensive patient M N begun *two weeks following* postpartum recovery from severe pre eclampsia This patient received only 200 mg of ACTH

sponse to TEAC became entirely pressor and remained so for several days despite discontinuance of the drug *The sustained nature of the* response despite the stopping of the drug resembles closely some recent experiences we have had in similar studies with cortisone [5]

Other Vascular Effects

In addition to the changes just demonstrated other vascular effects were noted in these patients In all instances a booming quality was noted in the blood pressure sounds within 24 hours after the start of ACTH The pulse volume increased and pulse pressure widened In two of the patients who had ballistocardiographic studies an *increase in stroke volume occurred without significant alteration of* cardiac output In one of these patients the blood volume was measured repeatedly and no change was noted Two *patients* after three weeks of ACTH developed swollen pink fingertips which persisted until the drug was stopped It is presumed that the skin capillaries of the distal portions of the digits were widely dilated and congested for during this period the mere needle prick for a blood count produced an unusual gush of blood

SUMMARY AND DISCUSSION

The rise in TEAC floor within 24 hours after the start of ACTH occurred without evidence of water retention preceded the increase in urinary 17 ketosteroids and preceded or paralleled depression of the eosinophiles In three of the patients following discontinuance of the drug the TEAC floor fell temporarily to low levels paralleling sodium diuresis and other evidence of reduced adrenal activity While these same patients were receiving ACTH changing the sodium intake (from normal to restricted or restricted to normal) produced additional shifts in the height of the TEAC floor similar in direction and magnitude to the same maneuver without ACTH

The mechanisms involved in these blood pressure and TEAC floor changes cannot be fully explained These changes probably reflect an increase in arteriolar tone for although stroke volume was uniformly increased the cardiac output remained unchanged

It seems clear that when the vascular tree is freed from autonomic influences that ACTH has a striking and reproducible effect on vascular tone as reflected in the TEAC floor

Although these changes in blood pressure and TEAC floor with administration and withdrawal of ACTH are more dramatic they are similar in extent and direction to those produced by salting and desalting The alterations in control blood pressure likewise bear similarities to changes reported with sodium and desoxycorticosterone acetate[6] Thus it is not unlikely that sodium may play a role in this mechanism however the TEAC floors appear to rise too promptly in response to ACTH to reflect significant sodium retention Some enhancement of the effects of sodium by ACTH remains a possibility

Possible pituitary pressor effects of the ACTH have been considered in an effort to account for the changes demonstrated Such explanation now appears untenable since we have been able to produce similar phenomena in a normotensive and a hypertensive patient with cortisone[5] Thus it would seem that the adrenal gland probably holds the clue to this mechanism and certain speculations are then possible It may be that the administration of ACTH causes the adrenal to elaborate some pressor substance or by adrenocortical stimulation causes potentiation of the effects of some pressor agent already present For example Raab[7] has recently shown that desoxycorticosterone acetate potentiates the pressor response to epinephrine and nor epinephrine In either event the result might be an alteration in the vascular response to autonomic blockade such as we have demonstrated

It might be further mentioned that the injection of TEAC is known to produce some potentiation of the response to epinephrine[8] However benzodioxane studies in two of the patients reported here remained the same during ACTH administration as during control observations

Finally although an exact explanation of mechanisms cannot yet be made the results point up anew the possible involvement of adrenal factors in hypertension

In conclusion the data suggest

1 ACTH administration and shifts in sodium intake significantly alter the blood pressure and TEAC floor These changes appear to be additive and are reflected mainly in the non neurogenic or humoral component of vascular tone

2 The TEAC floor appears to be a sensitive indicator of the adrenocortical activity produced by ACTH The response precedes other metabolic effects and either precedes or parallels depression of the eosinophiles

3 Certain other vascular responses to ACTH have been described These include increase in stroke volume with concomitant increase in pulse volume and pulse pressure and swelling and hyperemia of the fingertips and nailbeds The influence of sodium on these responses was not determined

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DISCUSSION

DR NATHAN SHOCK (Baltimore Md) The work I shall report was carried out in our laboratories by Dr Leroy F Duncan Dr David H Solomon (metabolism studies) and Dr Milton Landowne (cardiovascular measurements) Dr K Dobriner has kindly made the analyses for 17 ketosteroids and formaldehydogenic steroids in the urines of the patients studied

Our research was designed to study differences in response to chronic administration of ACTH that might be attributed to age Four patients have been observed two were 30 years of age and two were 63 and 65 years of age respectively The patients were carefully screened to eliminate the presence of diseases known to or suspected of altering the responsiveness to adrenal cortical stimulation or the end-organ reactions thereto After a 15 day control period each subject received 100 mg ACTH per day for 12 days Observations were continued for 18 additional days All patients were maintained on a uniform analyzed diet In addition to balances for nitrogen sodium potassium calcium phosphorus and chloride further observations were made such as urinary glucose uric acid formaldehydogenic and 17 ketosteroids blood eosinophile counts sedimentation rate arterial and venous pressures cardiac size electrocardiograms and cardiovascular response to exercise The metabolic responses to chronic administration of ACTH were in accord with previous findings Sodium chloride and water were retained The ratio of sodium to chloride retention exceeded the accepted extra cellular ratio of these ions Two subjects (one old and one young) showed marked responses one subject (young) showed intermediate responses One aged subject responded with only a slight nitrogen loss and low urinary glucose uric acid formaldehydogenic and 17 ketosteroid excretion although his electrolyte retention was as large as that of the other subjects In the four subjects tested individual differences in metabolic responsiveness did not seem to be related to age

Blood pressure (average of 7 consecutive determinations) and pulse rate (average of 3 one minute counts) were measured daily before the patient arose in the morning Average values for the last 9 days of the control ACTH and recovery periods respectively are presented for six subjects in Table I and demonstrate the rise in sys

Table I

EFFECT OF CHRONIC ADMINISTRATION OF ACTH ON BASAL BLOOD PRESSURE AND PULSE RATE

Subject	Age	Control Periods (9 days)		During Last 9 Days of ACTH Administration		Post ACTH (Last 9 Days of Recovery)	
		Blood Pressure mm Hg	Heart Rate per min	Blood Pressure mm Hg	Heart Rate per min	Blood Pressure mm Hg	Heart Rate per min
R K	31	118/74	65	128/70	45	116/70	66
B R	32	94/53	70	105/59	61	95/54	67
W M	65	117/66	68	141/71	53	123/69	69
W R	63	91/57	63	97/58	61	93/55	67
H C	64	143/74	64	157/74	57	136/71	67
J M	72	103/42	56	104/44	53	104/42	54
Mean		111/63	64 3	122/63	55 0	111/60	65 0

tolic pressure and the bradycardia observed during ACTH administration

The cardiovascular response to standard exercise (Master s two step test) was also determined Electrocardiograms were recorded and the heart rate was determined from five consecutive beats The changes in heart rate after exercise are shown in Table II The values are averages of at least two tests During the control period resting heart rates (not basal) average 73 3 beats per minute with an average increase to 96 5 beats per minute following exercise During the administration of ACTH resting heart rates dropped to an average of 63 8 per minute with an increase to 104 per minute after exercise Thus during ACTH administration the increment in heart rate

Table II

EFFECT OF CHRONIC ADMINISTRATION OF ACTH ON HEART RATE RESPONSE TO STANDARDIZED EXERCISE

Subject	*Age (years)*	*Control Periods*		*During ACTH Administration*		*Post ACTH (2 weeks)*	
		Resting	*After Exercise*	*Resting*	*After Exercise*	*Resting*	*After Exercise*
BR	32	72	88	66	93	72	82
WM	65	71	94	54	108	72	95
WR	63	73	90	66	94	73	82
HC	63	77	114	73	121	77	107
Mean		73 3	96 5	63 8	104 0	73 5	91 5

after exercise was greater in all subjects than before or after the hormone treatment Resting heart rates as well as increments after exercise returned to pre treatment levels within the two week period after the administration of ACTH was stopped (average 73 5) The results of these and other measurements we have made are consistent in indicating the cardiovascular stress which ACTH places upon the normal subject

DR RALPH GOLDMAN (Veteran s Administration Center University of California at Los Angeles California) Our interest in cardiovascular responses to ACTH has a different basis than that of Dr Brust and his associates We are interested in finding out the relationship of pituitary adrenocortical mechanisms to the formation of edema in congestive heart failure To this end we have balance data on three patients with arteriosclerotic heart disease and congestive failure All patients were maintained on a balance diet containing 500 mg of

sodium daily The first patient T M (Figure 8) showed no increased sodium retention after the administration of ACTH Chloride balance was also unchanged but there was an initial potassium diuresis lasting one day The black bars in the figure rising above the intake line represent additional sodium during sodium thiocyanate and sodium para aminohippurate administration Upon discontinuance of ACTH the patient had a massive sodium diuresis followed one day

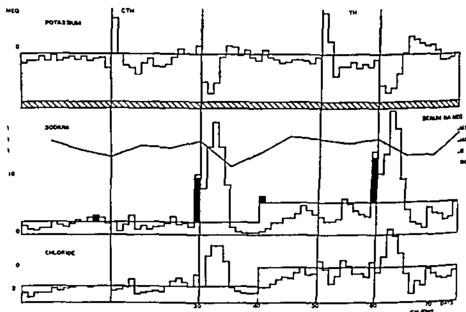


Fig 8 (Patient T M) *Metabolic Balance Data* The upper horizontal line represents the daily dietary intake The diagonally hatched area represents fecal excretion The step like horizontal lines represent the summation of fecal and urinary excretion when they extend above the dietary intake they indicate negative metabolic balance and when they fail to reach the intake level they represent positive metabolic balance The fecal sodium and chloride excretions were too negligible to be graphed The smaller black bars lying on the sodium intake line represent sodium administered as sodium thiocyanate whereas the longer black bars represent the sodium administered as sodium para aminohippurate

later by a somewhat smaller chloride diuresis It is estimated that one fourth to one third of the available sodium calculated on the basis of extracellular space was lost during this diuresis and that this greatly exceeded any retention during the preceding 30 days This apparently represented depletion of body sodium as is indicated by the fall in serum sodium concentration At the same time there was a marked potassium retention Starting five days after ACTH withdrawal there was a rebound retention and the sodium excretion

reached extremely low levels apparently in an attempt at repletion At 40 days the sodium intake was increased to approximately 1400 mg daily and the cycle of ACTH administration and withdrawal was reproduced

Figure 9 represents an attempt to correlate actual weight changes

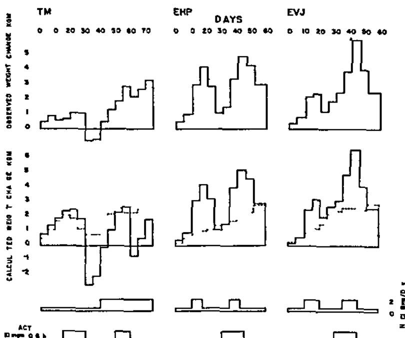


FIG 9 (Patient T M) Comparison of weight changes with weight changes calculated by metabolic data The upper figures represent the observed weight changes The solid lines in the lower figures represent calculated weight gains of tissue and extracellular water The tissue gain is calculated on the basis of grams of nitrogen balance multiplied by 27 while the extracellular water is calculated by dividing the sodium balance by the serum concentration of sodium at the time The dotted lines represent weight gain due to tissue changes apart from edema This is calculated on the basis of grams of nitrogen balance multiplied by 32 which includes the extracellular fluid normally contained by the tissue The constants are those of Reifenstein Albright and Wells [1]

with theoretical weight changes calculated from nitrogen and sodium balances using the constants of Reifenstein Albright and Wells The upper figure in each pair represents observed weight the dotted line in the lower figure represents weight change attributable to nitrogen balance alone whereas the solid line in this figure represents the weight change attributable to both nitrogen and sodium balances In the first patient T M it will be noted that the calculated data

demonstrate that when he was severely depleted of sodium he was capable of extreme sodium retention greater than that produced by ACTH

The second patient E H P developed edema even while on his basal 500 mg sodium diet Increasing his dietary intake of sodium by 1500 mg per day resulted in retention of the entire increment of sodium and the actual weight gain paralleled the theoretical ACTH failed to alter this cycle and there was no other evidence of ACTH response It will be appreciated that this patient retained almost his entire sodium intake without the usual evidences of adrenocortical activity

The third patient F B J was subjected to a similar regimen As can be seen from the observed and calculated weight changes there was no significant sodium retention on the basal diet but increasing the sodium caused sodium retention for six or seven days when the urinary sodium increased and sodium balance was again achieved When ACTH was administered the patient retained sodium on the basal diet and upon administration of additional sodium the excess was retained and there was no return to equilibrium after ten days Administration of ACTH caused an eosinophil drop and a negative nitrogen balance and indicated that further ACTH response was possible even though the patient was in congestive failure and sodium retention could be produced by fluctuations in the sodium load

SUMMARY

These three patients demonstrated inconstant responses to ACTH administration which when they did occur were of the expected type There was no evidence from this data that renal retention of sodium during congestive failure depended upon the stimulation of the adrenal cortex by ACTH

DR PETER H FORSHAM What is the role of the adrenal in hypertension? Dr Brust s presentation touched upon this subject by cutting out the sympathetic component of the hypertensive response Drs Thorn Merrill and their associates at the Peter Bent Brigham Hospital are engaged in the study of the effect of bilateral adrenalectomy on hypertension in man and are thus approaching the problem from the other extreme

Of three patients subjected to such a procedure so far one died of coronary infarction within one week after surgery and the two surviving patients have now gone two and three months postoperatively The blood pressure did show a tendency to fall over a period of a week following operation on 25 mg of cortisone acetate a day

given intramuscularly or by mouth in divided doses This dose with adequate food intake prevented hypoglycemia and weakness However with such therapy alone there was a continued fall in blood pressure to levels incompatible with normal physiological function The addition of between 1 and 3 mg of desoxycorticosterone tended to stabilize the blood pressure This hormone appeared to control blood pressure rather significantly since giving 5 or 10 mg a day led to definite re establishment of the preoperative hypertension

Such finding raises the perennial question as to whether the hypertensive effect of desoxycorticosterone is due to salt and water retention with an increase in blood volume or to a direct reaction on the musculature of the arteriolas The findings of Dr Brust suggest that this direct vascular effect is more important than that on the blood volume and with this the findings of Dr Lewis Dexter and his group of the Peter Bent Brigham Hospital are in complete agreement He did some studies on cardiac output and peripheral resistance before and following over treatment with ACTH accompanied by the establishment of a hypertension of 180/120 mm Hg It turned out that while the cardiac output was not increased peripheral arteriolar resistance increased 100% during the period of ACTH over treatment From the work of Selye it would appear most likely that desoxycorticosterone like factors are responsible for this increase in peripheral resistance under excess ACTH We would thus agree with the early suggestions by Perrera that the direct effect on the vasculature is an important factor in the hypertensive activity of adrenal cortical hormones

DR JEROME W CONN I have one comment in that respect We are studying a patient at the present time who has Addison s disease and whose blood pressure has run between 80 and 100 systolic After 10 days of 25 milligrams of cortisone per day without any added desoxycorticosterone the blood pressure has risen to 170 over 110

There is no knowledge of pre existing hypertension before the Addison s disease

DR IRVINE MCQUARRIE We found some years ago in a 14 year old diabetic boy who craved salt that whenever he took 80 or 90 grams of sodium chloride a day to satisfy his taste while on a low potassium standard diet his blood pressure would go to 185 or 190 systolic and 112 or 115 diastolic When maintained on an ordinary diet without extra sodium chloride his blood pressure was normal We found that potassium chloride had an antagonistic effect to the excessive sodium intake One milli equivalent of potassium was found to antagonize about three milli equivalents of sodium

When the patient was given potassium chloride alone while on a standard low sodium diet the blood pressure fell even slightly below his normal level The same type of response was obtained subsequently in other normotensive diabetic children who were not salt cravers The attention of nearly everybody appears to be on sodium alone but I think we should always keep in mind this relationship between sodium and potassium

Here in these situations where ACTH is being given the serum potassium falls while sodium is being retained So small amounts of sodium can do quite big things if the potassium content of the diet is very low Both basic elements should be taken into account

Of course it is recognized that the adrenal steroids play an important associated role as indicated by the work of Selye Perera and others

DR FREDERICK C BARTTER I would like to present an experiment which supports Dr Brust s contention that vascular responses to ACTH may *not* be dependent upon an electrolyte effect

Metabolic studies were carried out over a 200 day period in a patient with carcinoma of the breast ACTH was administered for three periods lasting 30 24 and 21 days the dosage varying from 50 to 100 mg a day There was a rise of blood pressure during all three courses of ACTH Whereas there was an associated weight gain and sodium retention during the first two courses the weight gain and presumably sodium retention were prevented during the last course by sodium restriction (9 mEq/day) ACTH clearly had a hypertensive effect that was not dependent on its sodium retaining effect in this case

DR MARVIN F LEVITT I would just like to make one point with reference to these TEAC floors and the change in blood pressure in the absence of any obvious over all sodium balance change

We have one patient maintained on a rice diet in whom there was a 20 per cent increase in the isotonic extra cellular volume 36 hours after the first injection of cortisone

Although there may be no obvious changes in over all sodium balance there may be a shift very rapidly and I wonder if that has any relation to the blood pressure changes

DR ALBERT A BRUST Many of the discussers remarks do not require any further comment by myself

With respect to Dr Goldman s observations we should mention that we too were unable to correlate blood pressure and the tetra

ethylammonium chloride floor changes with changes in body weight during ACTH administration

With regard to Dr Forsham s comments we are all extremely interested in the bilateral adrenalectomy studies The responses he mentioned i e the blood pressure not coming to normal after bilateral adrenalectomy I believe are consistent with results that have been noted by Dr Page s group in a small number of patients in Cleveland

Dr Forsham mentioned that blood volume changes were not observed in Dr Dexter s patient We did blood volume studies in one of our four patients at one week intervals and found no appreciable change

Dr Conn s comments about Addison s disease may be pertinent to our observations In a patient with adrenal insufficiency who was desalted the blood pressure response to tetraethylammonium chloride was observed to fall to shock levels and to rise again when diet sodium was increased The low TEAC floors in this Addisonian patient in the desalted state were quite comparable to those we have demonstrated here after the discontinuance of ACTH

We would certainly agree with Dr McQuarrie in terms of the possible importance of potassium When we talk about sodium and potassium we often don t know which is which—whether we re measuring an excess of one or a deficiency of the other Serum levels obviously do not reflect significant changes in tissue concentrations There is some suggestion from our data that shifts in potassium content may be equally as important as shifts in sodium content as far as vascular tone is concerned In that connection Dr Leavitt s observations of the shift in extra-cellular space would appear most appropriate

20

The Effects of ACTH on the Electrolyte Content of Various Body Tissues*

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NEW YORK AND CORNELL UNIVERSITY MEDICAL COLLEGE

Adrenocortical hyperfunction whether spontaneous or induced may be associated with hypopotassemia hypochloremia and metabolic alkalosis It might be inferred from Darrow s and his colleagues work in animals[1] that these disturbances in electrolyte and acid base equilibrium would be associated with a tissue potassium deficit and increase in sodium Kepler et al have indeed shown this to be the case in Cushing s syndrome[2,3] We have attempted by means of balance studies tissue analyses and dilution techniques to obtain an integrated description of the electrolyte shifts which occur following administration of ACTH or cortisone Since the tissue changes resulting from induced adrenal cortical hyperfunction are of more or less acute nature it was also of interest to compare them with tissue changes in Cushing s syndrome which result from a physiologic disturbance of long duration

The balance study reported in this paper was done on a 42 year old male (L S) with follicular lymphosarcoma who had marked lymphadenopathy and splenomegaly (figures 3-6 Table I) He received ACTH 100 mg daily for 18 days during which time there was marked shrinkage of the enlarged spleen and lymph nodes and development of hypopotassemia hypochloremia and metabolic alkalosis Skeletal muscle and lymphoid tumor biopsies for analyses of Na K Cl P and N were done before ACTH was started and after 18 days of ACTH The results of these tissue analyses and of similar analyses obtained from two patients who received cortisone from a patient

* This study was supported by grants from the U S Public Health Service C 925(C2) jointly by the Office of Naval Research Contract N8 onr 66801 and the Atomic Energy Commission the Damon Runyon Cancer Research Fund and the American Cancer Society

† Damon Runyon Senior Clinical Research Fellow

‡ Atomic Energy Commission Post Doctoral Fellow in the Medical Sciences of the National Research Council

COMPARISON OF PATIENT S MUSCLE ERYTHROCYTE AND LYMPHOID TUMOR ANALYSES WITH THOSE OF A CORTISONE TRE TED PATIENT PATIENTS WITH CUSHING S SYNDROME AND NORMAL PATIENTS

	NAME	DIAGNOSIS	TISSUE	TREATMENT & DURATION	OUND VALUES					DERIVED DATA				WATER DISTRIBUTION ‡		
					$[N]_t$	$[P]_t$	$[K]_t$	$[N]_t$	$[Cl]_t$	$[K]_i$	$[N]_i$	$(K)_i$	$(N)_i$	$(H_2O)_t$	(H_2O)	$(H_2O)_i$
					%	%	mEq	mEq	mEq	mEq*	mEq*	mEq/L†	g/L		*	
1	L S	FOLLICULAR LYMPHOSARCOMA	MUSCLE (PECTORAL)	BEFORE ACTH AFTER 1 8gm/18 DAYS	136 7 145 9	8 73 7 8	408 4 382 9	137 2 166 0	87 5 110 8	405 2 379 8	21 9 33 5	142 4 115 7	7 7 4 1	784 813	169 199	615 614
2	N B	CHR LYMPHATIC LEUKEMIA		BEFORE CORTISONE AFTER 2 4gm/18 DAYS	247 1 141 5	7 44 8 52	417 7 402 7	153 6 176 3	67 9 112 3	412 8 397 7	36 8 36 3	131 0 125 0	11 7 11 4	798 806	161 190	637 616
3	R C	HODGKIN S DIS		AFTER CORTISONE 6 0 gm/30 DAYS	136 1	5 68	323 7	207 9	139 7	318 2	31 7	103 8	10 3	813	230	573
4	G R	CUSHING S SYND		NONE	134 8	5 21	314 0	236 8	152 9	309 9	25 3	126 0	10 3	800	308	492
5		CUSHING S SYND KEPLER (?)		NONE	244 0	7 63	373 0				50 0					
		*NORMAL MEAN § S E OF MEAN			144 9 3 1	7 90 27	400 7 10 4	142 0 13 9	97 4 1 8	400 6 5 8	33 9 6 3	134 3 4 1	11 4 2 1	791 4	169 5	622 8
1	L S	FOLLICULAR LYMPHOSARCOMA	ERYTHRO CYTES	BEFORE ACTH AFTER 1 8gm/18 DAYS			96 2 86 4	14 7 46 0	56 9 53 1							
4	G R	CUSHING S SYND		NONE			88 0	28 5	49 7							
		NORMAL MEAN △ S E OF MEAN					100 4 2 1	18 1 2 8	54 1 1 6							
1	L S	FOLLICULAR LYMPHOSARCOMA	LYMPHOID TUMOR	BEFORE ACTH AFTER 1 8gm/18 DAYS	145 2 140 7	20 87 20 54	592 8 513 1	208 8 272 6	282 7 284 2							
2	N B	CHR LYMPHATIC LEUKEMIA		BEFORE CORTISONE AFTER 2 4gm/18 DAYS	147 0 146 8	21 36 20 88	640 4 446 3	214 0 349 6	240 9 265 0							
		C BREAST (MASTECTOMY)	NORMAL LYMPH NODE	NONE	143 7	14 75	433 0	269 4	180 2							

SUBSCRIPTS T TOP OF CHART t SIGNIFIES TOTAL TISSUE CONTENT i IS INTRACELLULAR CONTENT AND IS EXTRACELLULAR CONTENT RESPECTIVELY

* PER KILOGRAM DRY FAT-FREE TISSUE OR PER LITER PACKED ERYTHROCYTES

† PER LITER OF INTRACELLULAR WATER

‡ PER KILO RAW WET FAT FREE TISSUE

§ SIX HOSPITAL PATIENTS NOT ACUTELY ILL AND WITH NO EVIDENCE OF ELECTROLYTE DISTURBANCE

△ FIVE HEALTHY ADULTS

* AVERAGE OF THREE UNTREATED PATIENTS

S E STANDARD ERROR OF MEAN = $\sqrt{\frac{\Sigma \Delta^2}{n(n-1)}}$ (MEANS FOR DERIVED DATA DO NOT INCLUDE THREE PATIENTS WHOSE DATA WERE INSUFFICIENT TO MAKE THE CALCULATIONS)

Fig 1 Comparison of Patient s Muscle Erythrocyte and Lymphoid Tumor Analyses with those of a Cortisone Treated Patient Patients with Cushing s Syndrome and Normal Patients

Table I

SUMMARY OF TOTAL AND INTRACELLULAR BALANCES OF SODIUM POTASSIUM AND CHLORIDE

Period No	Na mEq		K mEq		Cl mEq
	Total	*Intra C*	*Total*	*Intra C*	*Total*
CONTROL 1-2	−68	0	−31	0	−50
ACTH 3-8	+274	+136	−696	−213	+127
CONTROL 9-10	−885	−190	+171	+265	−543
11	+101	−28	−22	−2	+103

with Cushing s syndrome and from six normal patients are summarized in Figure 1

Dilution studies with radioactive sodium (Na^{24}) and bromide to determine the body content of sodium and chloride were carried out in two patients who had received ACTH for 10 and 18 days and who had demonstrated marked sodium chloride and water retention The studies were done just before ACTH was stopped and were repeated two weeks later after diuresis was complete Similar studies were done in a patient with Cushing s syndrome and in five normal adults The results are summarized in Figure 2

The methods and calculations used in the balance studies tissue analyses and dilution techniques have been described in detail elsewhere[4]

BODY SODIUM AND CHLORIDE CONTENTS DETERMINED BY DILUTION TECHNIQUES COMPARISON WITH BALANCE DATA AND WITH DATA FROM NORMAL ADULTS AND A PATIENT WITH CUSHING'S SYNDROME

	SODIUM			CHLORIDE		
	Σ Na m eq/kg	Δ Na^{24} m eq	BALANCE m eq	Σ Cl m-eq/kg	Δ Br m eq	BALANCE m eq
K B						
ACTH	40 1			37 5		
POST ACTH	36 9	290	-398	33 1	-210	-165
F T						
ACTH	70 0			64 8		
POST-ACTH	50 4	-1640	-1607	49 7	-1261	1045
CUSHING'S SYND	44 1			32 3		
NORMAL MEAN	38 5			28 9		
S E OF MEAN	1 4			3 4		

Σ TOTAL CONTENT

Δ DIFFERENCE IN TOTAL CONTENT PRE AND POST-DIURESIS

FIG 2 Body Sodium and Chloride Contents Determined by Dilution Techniques Comparison with Balance Data and with Data from Normal Adults and a Patient with Cushing s Syndrome

EXPERIMENTAL RESULTS

1 Balance Study and Tissue Analyses in a Patient Receiving ACTH

The balance studies on patient L S demonstrate that during the ACTH period there was a large loss of nitrogen and phosphorus (figure 3) The loss of phosphorus was markedly in excess of that expected from the nitrogen and calcium balances (broken line) if normal tissue had been destroyed We have previously shown that lymphoid tumor has a high phosphorus content with respect to muscle (Figure 2) and that destruction of lymphoid tumor accounts for the discrepancy [55] The tissue analyses show a slight fall in muscle phosphorus content after ACTH but this is not sufficient to account for the excess loss No significant change in tumor phosphorus content was noted Utilizing the ratios of nitrogen to phosphorus in muscle and lymphoid tumor it can be calculated as a first approximation from the balance data that about 2 kilograms of tumor and 6 5 kilograms of muscle were destroyed [5]

During the period when ACTH was given there was a fall in serum potassium to 2 95 mEq L (figure 4) The serum carbon dioxide content and pH rose moderately indicating metabolic alkalosis A fall in serum chloride (figure 5) was also observed

The potassium balance (figure 4) shows a large loss of potassium when ACTH was given which at first was in marked excess of the amount one would have expected from the nitrogen (broken line) if normal tissue had been destroyed The ratio of potassium to nitrogen in tumor is slightly higher than that in muscle but would not account for the excess loss of potassium even if nothing but tumor tissue had been destroyed There was a total loss of 213 milliequivalents of potassium in excess of nitrogen during the ACTH period (see Table I Figure 6) indicating presumable intracellular depletion The tissue analyses reveal reductions in potassium content of 6% 10% and 13% in muscle erythrocytes and lymphoid tumor respectively (Figure 1) Both the muscle intracellular potassium content and concentration in intracellular water were reduced The tissue analyses thus confirm the changes predicted from the balance

The sodium and chloride balances (figure 5) show a marked retention of these electrolytes when ACTH was given The tissue analyses likewise reveal an increase in muscle sodium chloride and extracellular water content The increase in chloride and extracellular fluid is sufficient to account for the rise in sodium The tumor and erythrocyte sodium contents were markedly increased

The intracellular sodium balance was computed by assuming that

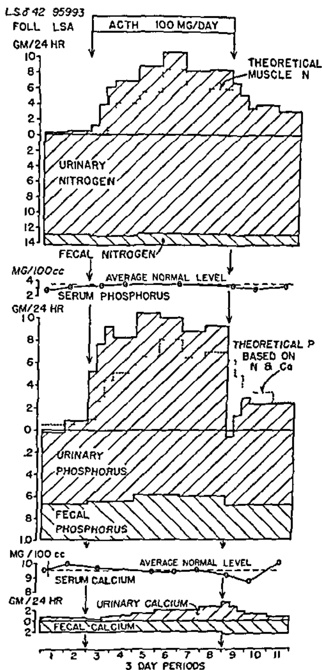


FIG 3 The effects of ACTH on the nitrogen phosphorus and calcium balances and on the serum phosphorus and calcium concentrations

The balance charts are constructed by plotting the intake downwards from the zero line and by plotting upwards from the intake first the fecal then the urinary excretion *If the sum of the excretions coincides with the zero line balance is indicated if they rise above the balance is nega*

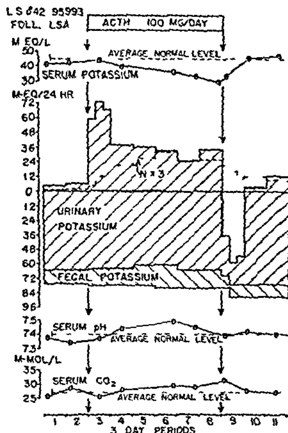


FIG 4 The effects of ACTH on the potassium balance and on the serum potassium concentration carbon dioxide content and pH The broken line represents the potassium balance that would have been expected from loss or gain of normal tissue based on the K N ratio (mEq gm) of 3 in the patient s muscle

chloride is exclusively extracellular and subtracting from the sodium balance the change in extracellular fluid sodium content These calculations show that during administration of ACTH there was an intracellular retention of 136 milliequivalents of sodium (see Table I

tive and if they fall below leaving a clear space the balance is positive The ordinates of the nitrogen phosphorus and calcium charts are so chosen that equal heights represent the approximate ratios at which these elements exist in normal protoplasm and bone that is 15 gm of nitrogen to 1 gm of phosphorus (protoplasm) and 2 gm of calcium to 1 gm of phosphorus (bone) The sum of the nitrogen and calcium balances gives the theoretical phosphorus balance (broken line) that is the phosphorus balance that would be predicted from changes in protoplasm based on nitrogen plus changes in bone based on calcium

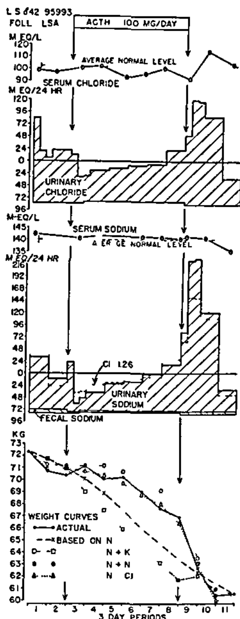


FIG 5 The effects of ACTH on the sodium and chloride balance on the serum chloride and sodium concentrations and on the actual and theoretical weight curves

and figure 6) Muscle analysis revealed no increase in intracellular sodium but the intracellular tumor sodium presumably increased rather markedly since the tumor chloride content remained relatively unchanged while the total sodium content increased in the same order of magnitude as the decrease in potassium It will be noted

that the chloride content of the tumors shown in Figure 1 actually exceeds the sodium content Chloride therefore must exist within the cells of these tumors in substantial quantity resembling in this respect erythrocytes

After the first 6 days of ACTH the urinary sodium and chloride excretion rose gradually until during the last three days there was actually a loss of 51 and 65 milliequivalents respectively (figure 5) When ACTH was stopped there was a prompt diuresis of sodium chloride and water The loss of sodium and chloride however ex

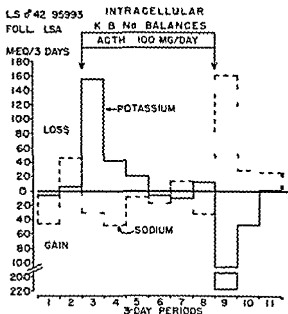


Fig 6 The effects of ACTH on the intracellular balances of potassium and sodium

ceeded by a factor of three to four the preceding retention (Table I) suggesting the possibility of adrenal cortical hypofunction during the immediate post ACTH periods The last period shows a sharp reten tion of sodium and chloride suggesting that recovery of cortical func tion was prompt The balance data during the post ACTH periods also indicate movement of potassium back into the cells while so dium migrated out

The actual and theoretical weight curves (figure 5) further em phasize the opposite movements of potassium and sodium demon strated by balance studies (Table I and figure 6) and tissue analyses (Figure 1) The weight curve predicted from the nitrogen and chlo ride balances most closely approximates the actual weight during the

ACTH period The weight curve predicted from nitrogen and potassium however falls below the curve predicted from nitrogen loss alone indicating intracellular potassium depletion while the curve predicted from the nitrogen and sodium balances lies substantially above the nitrogen plus chloride curve showing retention of sodium above that which could be accounted for by chloride presumably due to migration of sodium into the cells The tissue analyses suggest that the increase in intracellular sodium occurred in tissues other than muscle such as erythrocytes and lymphoid tumor

2 Tissue Analyses in Patients Receiving Cortisone and in Cushing s Syndrome

A patient M B with chronic lymphatic leukemia who received cortisone for 18 days demonstrated tissue changes similar to those described above (Figure 1) except that the fall in muscle potassium was *somewhat less No significant increase in muscle intracellular so*dium was found This patient did not develop hypopotassemia metabolic alkalosis or hypochloremia In contrast to the small changes in the muscle were a 30% decrease in tumor potassium and a 63% increase in tumor sodium The increased tumor sodium probably represents largely an intracellular increment As in the previous patient the tumor chloride remained relatively constant and the fall in potassium was of the same order of magnitude as the increase in sodium There was a slight increase in muscle phosphorus and no significant change in tumor phosphorus

Patient R C on the other hand who received cortisone in larger dosage for 30 days demonstrated a marked lowering of muscle potassium compared to the normal mean No increase in intracellular sodium was found Her serum potassium fell to 2 2 mEq/L There was a mild metabolic alkalosis but no hypochloremia There was also a marked reduction of muscle phosphorus Tissue extracellular water was also markedly increased at the expense of intracellular water without significant increase in total tissue water

Patient G R with Cushing s syndrome of 6 years duration had hypopotassemia hypochloremia and metabolic alkalosis The alterations in the muscle electrolyte pattern and water distribution were similar in every respect to those in the patient just described (R C) but more pronounced Though Kepler s patients with Cushing s syndrome had muscle phosphorus contents approximating our normal values (Figure 1) they averaged 24% lower than his stated normal while the potassium contents averaged 22% below his normal values The muscle sodium (presumably intracellular) was increased in only one of three patients[3]

3 Dilution Studies with Radiosodium (Na^{24}) and Bromide

The two patients in whom dilution studies were done during administration of ACTH demonstrated sodium and chloride contents expressed in milliequivalents per kilogram of body weight which were above the values for normal adults and are consistent with the increased tissue sodium and chloride contents shown in Figure 2 Neither of the patients developed hypopotassemia or metabolic alkalosis In both patients a large diuresis of sodium chloride and water occurred promptly on cessation of ACTH The determinations of body sodium and chloride content following diuresis showed decreased contents which are in good agreement with the losses determined from the balance data considering the over all error of the method (±5%) These results have served to establish the validity of the sodium and chloride balances by ruling out any appreciable imperceptible losses

The radiosodium and bromide studies in the patient with Cushing s syndrome (C R) demonstrated a sodium and chloride content above normal as did the muscle analyses (Figure 1)

GENERAL DISCUSSION

The failure to find an elevated intracellular muscle sodium content in the presence of potassium deficit and metabolic alkalosis in our patients is not consonant with Darrow s findings in potassium deficient animals[3] Darrow s observations indicate that there is a predictable correlation between the intracellular potassium and sodium content of muscle and serum bicarbonate concentration the intracellular sodium content and serum bicarbonate concentration rising as the intracellular potassium content falls

Two possible explanations for this discrepancy are 1) The intracellular sodium content of tissues other than muscle may increase as was demonstrated in our patient s erythrocyte and tumor tissues 2) Three of the patients studied showed falls in muscle phosphorus content two of them marked These falls if calculated in terms of milliequivalents of inorganic phosphate ion at pH 7 4 exceed the decreases in potassium content It appears as though depletion of intracellular anion phosphate may have eliminated the requirement for replacement of lost cation potassium by sodium

In tumor tissue on the other hand in which there was no decrease in phosphorus content and a large fall in the potassium content an apparent increase of intracellular sodium occurred which was of similar magnitude to the fall in potassium In muscle there was a depletion of both intracellular anion and cation An interesting conse

quence of this depletion is a transfer of water from the intracellular to the extracellular compartment in order to maintain normal intra cellular osmolar concentrations This is demonstrated by the data on patients R C and G R in Figure 1

SUMMARY AND CONCLUSIONS

1 Electrolyte analyses of muscle lymphoid tumor and erythrocytes were made in a patient who was given ACTH while on a bal *ance regime and who developed hypopotassemia hypochloremia and* metabolic alkalosis There is good correlation between the changes predicted from the balance data and those actually found in the tissues

2 The tissue electrolyte changes found were

a Muscle
- i Extracellular sodium increased
- ii Extracellular chloride increased
- iii Extracellular water increased
- iv Intracellular potassium decreased
- v Intracellular phosphorus decreased
- vi Intracellular sodium *not* increased

b Erythrocytes
- i Sodium increased
- ii Chloride unchanged
- iii Potassium decreased

c Lymphoid tumor
- i Sodium increased
- ii Chloride unchanged
- iii Potassium decreased
- iv *Phosphorus unchanged*

3 Similar tissue changes were found in two patients who received cortisone except that they were much more profound in one patient who received high doses for 30 days The latter patient demonstrated in addition a marked fall in muscle phosphorus no elevation of intra cellular sodium and a large transfer of water from the intracellular to the extracellular compartment

4 *A patient with Cushing s syndrome exhibited tissue electrolyte* and water alterations essentially the same as those seen in the patient who received high doses of cortisone but more marked in degree

5 Dilution studies revealed elevated sodium and chloride con tents in patients receiving ACTH and in a patient with Cushing s syndrome The loss in sodium and chloride following withdrawal of ACTH has been measured both by balance and dilution studies The two methods are in *essential agreement*

6 It is suggested that the failure to find an elevation of intracellular muscle sodium content in the presence of potassium deficit and metabolic alkalosis in patients receiving ACTH or cortisone may be attributed to loss of muscle phosphorus

7 The balance data also show that large losses of sodium chloride and water occurred when ACTH was stopped These losses exceeded by factors of three to four the amounts retained during administration of ACTH suggesting a short period of adrenal cortical hypofunction after ACTH was discontinued

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DISCUSSION

DR MARVIN F LEVITT The use of sodium and chloride as measures of extracellular volume have been subject to some doubt recently It has been demonstrated that the space of distribution of inulin which has a molecular weight of 5 000 and which is apparently excluded from the cell is significantly smaller than the simultaneous sodium or chloride space

In Figure 7 are shown the data on a patient maintained on the salt free rice diet The extracellular volume was measured with the

inulin method serially during a course of cortisone treatment Note that there is a progressive augmentation of relatively isotonic extra cellular volume of about 40% despite the rigid sodium and chloride restriction This change reached its peak after eight or nine days of therapy and then subsided despite the continuation of treatment

This one patient is typical of four who have been similarly stud

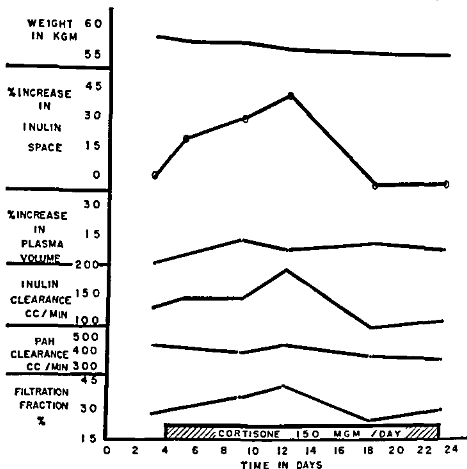


Fig 7

ied with cortisone or ACTH In each instance the changes in the extracellular compartment are not reflected in simultaneous water chloride or sodium balances Coincidentally plasma volume measure ments offer only a qualitative hint of the magnitude of the extra cellular volume changes At the time of maximal increase in extracel lular volume there is about a 10% increase in plasma volume

These changes imply that cortisone and ACTH produce a shift of sodium chloride and water into the measurable extracellular com

partment further that this shift reaches its peak after the eighth or ninth day of treatment and then spontaneously subsides despite the continuation of treatment

DR DAVID RUTSTEIN (Harvard Medical School Boston) There are two points which would merit discussion The first concerns the value of different substances in the measurement of various kinds of spaces in the body There are no data which indicate that any particular substance measures any specific anatomical space within the body When substances are injected for the measurement of space by the dilution technique the results obtained depend upon such factors as diffusibility binding tendencies and ability to penetrate cell membranes with the further assumption that the substances are neither metabolized nor excreted (or that a correction can be made for these latter factors)

Thus one measures physiological rather than anatomical space since the conditions under which these characteristics may change are not known It is important that reproducible results be obtained under the same experimental conditions It might be finally stated parenthetically that it may be even more important to measure physiological spaces rather than anatomical spaces

The second point concerns observations made in our laboratories on shifts in water balance occurring in normal individuals who were given 200 milligrams of cortisone per day for a period of approximately five days These subjects were kept on a fixed diet based on their usual sodium and chloride intake *

It was found that T 1824 space was increased from 10% to 35% during cortisone administration that the change in extravascular thiocyanate space was smaller than this and also smaller than that reported for inulin space by the previous discussant There was in addition a small increase in weight of the subjects during the experimental period and in the one case in which it was measured The increase in total body water as determined by the deuterium oxide technique paralleled the change in weight

DR LYTT GARDNER (Johns Hopkins Hospital and Johns Hopkins University School of Medicine Baltimore) I think Dr Elkel is to be complimented on getting into the meat of the subject in such a direct manner because so few people have bothered to confirm space data by tissue analysis His observations on phosphate are of the greatest

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* We wish to acknowledge the cooperation of Dr George W Thorn Physician in Chief and Miss Mary Kascht Research Dietitian Peter Bent Brigham Hospital for their assistance in providing the diets

importance because we are finally getting down into the energy transfer systems of the cell

One of the first experiments done on the effect of insulin on mineral metabolism showed that potassium and phosphate appeared to move into the cell I think that Doctor Eliel s observations on the movements of phosphate and potassium are of interest in the light of this early experiment

I would like to know if you have had any opportunity to fractionate tissue phosphate into the lipid the inorganic and the organic fractions in order to get a more definite idea of what is moving across the cell membrane

DR W H FISHMAN (Tufts College Medical School Boston) I would like to mention some of our experiences relating to the significance of phosphorus in the urine of patients treated with ACTH

In the patients which were studied in this last paper one is dealing with two phenomena One the lysis so to speak of the lymphosarcoma which will be reflected in the urine by the excretion of excess nitrogen and phosphorus and the other phenomenon is the catabolic effect of the ACTH on non tumor proteins

Our experiences have been limited to metabolic studies of four arthritic patients and one Hodgkins disease patient receiving ACTH and in these studies we do observe as has everyone else on high doses of ACTH a negative nitrogen balance but the amount of phosphorus which appears in the urine and the feces is not what one would expect on the basis of protein of the protoplasm being broken down to yield nitrogen and phosphorus in their accustomed ratios

As a matter of fact a relative deficit of phosphorus was observed in every one of five separate studies except for Dr Albright whose group have observed the opposite phenomenon in a case of panhypopituitarism in which there was a relative retention of both phosphorus and potassium during ACTH therapy Their results could be explained by the deposition of glycogen which is known to require phosphorus and potassium in very definite proportions for its formation

In view of the results of a reduced phosphorus content of muscle after ACTH and in view of our findings that the phosphorus does not apparently appear in the urine in the amounts that one would expect from the breakdown of protoplasm I think one has to look for some tissue other than muscle where apparently an excess of phosphorus is being utilized

DR JOSEPH E WARREN The problem of sodium retention and water balance during the initial response to ACTH is of extreme clinical

importance in patients with acute rheumatic carditis especially if they already have some congestive failure

We have routinely put such patients on a very low sodium intake but despite this several patients have shown a marked increase in respiratory distress during the first two or three days This orthopnea and tachypnea was out of all proportion to the maximal amount of fluid retention that could have been covered by the retained sodium

We postulated an increase in blood volume due to mobilization of extravascular fluid but did not anticipate such marked shifts across all membranes as Dr Eliel s data suggest

The practical problem in some patients therefore requires either a start with a lower dosage of ACTH until spontaneous diuresis sets in or the addition of mercurial diuretics very early in the regimen

DR LEONARD P ELIEL (New York N Y) Dr Levitt s data are extremely interesting and I ve been looking for a nice demonstration of that sort which suggests that chloride may actually occupy an intracellular position

Our data (Figure 1) also indicates that chloride may occupy an intracellular position This is certainly true in the case of erythrocytes which constitute a pretty large mass and in the lymphoid tumors in the particular patients we studied where the chloride content in fact exceeded the sodium content In this respect the lymphoid tumors are similar to erythrocytes It remains to be demonstrated however that chloride exists intracellularly in muscle in any appreciable quantity

Our data also indicates that there are transfers of water from the intracellular to the extracellular compartment without very marked change in the total tissue water which would be in accordance with Dr Levitt s findings

We have not claimed that the measurement of sodium and chloride content measures extracellular fluid volume We have interpreted these measurements more in the physiologic sense that Dr Rutstein discussed

As for Dr Gardner s question we did extract our tissues for neutral fat and for phospholipid and there were no consistent changes in the lipid phosphorus fraction

Dr Fishman commented that ACTH given to patients without lymphoid tumors results in smaller losses of phosphorus than one would expect from destruction of normal tissue Dr Bartter and his colleagues have suggested that this may be due to deposition of phosphorus with glycogen Our balance studies which reflect a large phosphorus loss due to tumor destruction do not reflect what happens in individual tissues they just show over all changes There could have

importance because we are finally getting down into the energy trans fer systems of the cell

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DR JOSEPH E WARREN The problem of sodium retention and water balance during the initial response to ACTH is of extreme clinical

21

Muscle Biopsy with Reference to Tissue Sodium and Potassium Determinations*†

Peter J Farago Isidore Rochlin Robert C Schilling Gordon F Vawter and S Howard Armstrong Jr

PRESBYTERIAN HOSPITAL OF THE CITY OF CHICAGO AND UNIVERSITY OF ILLINOIS COLLEGE OF MEDICINE CHICAGO

INTRODUCTION

With the introduction of hormones such as ACTH and cortisone attention has become focused even more sharply than ever before on the problem of electrolyte metabolism The flame photometer has made it relatively simple to obtain prompt and accurate determinations of serum sodium and potassium levels which often prove to be of great clinical value However the estimation of tissue electrolytes involves in most instances a complicated and cumbersome procedure which is usually confined to the physiology laboratory

Previous work has been done by other investigators in an attempt to simplify the task and apply the results to clinical usage[1] The difficulty with earlier methods has been

a The length of time involved in arriving at an answer

b The correction for fat which in itself is a time-consuming procedure and does not lead to clinical applicability and

c Differentiating intracellular and extracellular fluid on the basis of the chloride space when interpretation of the latter is fraught with so many theoretical and practical difficulties

METHOD

At present we are employing a method for the determination of tissue sodium and potassium which is rapid and easy The details of the procedure now in use are as follows

* This work was supported by funds from the U S Public Health Service (Grant No H 811) the Otho S A Sprague Memorial Institute of Chicago and the A D Thomson Fund of the Presbyterian Hospital

† We wish to express our gratitude to Dr John Dorsey and Dr Frank V Theis of the Attending Staff in Surgery and to Dr John Latimer of the Resident Staff for their collaboration in standardizing the techniques for these biopsies

been simultaneous glycogen and phosphorus deposition in some tissues with *major depletions in others* which could not be detected under the conditions of the experiment Furthermore a decrease in muscle phosphorus content does not necessarily mean there are decreases in other tissues In fact there might possibly be and probably are increases in other tissues

standards contain no HNO_3 but are otherwise identical with Procedure (a) standards Following routine analysis on the Beckman flame photometer the sodium and potassium contents are calculated Duplicates using either procedure agree within 3% The filtrate may be used for other determinations

REPRODUCIBILITY

Table I illustrates reproducibility by determinations on samples taken from a single piece of beef muscle at distances over 3 cms apart

Table I

TISSUE SODIUM AND POTASSIUM DETERMINATIONS OF BEEF MUSCLE

*Sample**	*Sodium (mEq/Kg of fresh tissue)*	*Potassium (mEq/Kg of fresh tissue)*	*Sodium Potassium (mEq/Kg of fresh tissue)*
I	35	98	133
II	34	100	133
III	36	99	135
IV	37	99	136
AVERAGE	35	99	134
RANGE	34–37	98–100	133–136

Four samples from various areas of the same muscle

RESULTS

This method has been applied to human muscle The values in Table II are results of human biopsies obtained at time of operation from surgical patients who were not in any obvious electrolyte imbalance These patients received a general anesthetic They represent different muscles age groups and sexes A few analyses were done on post mortem tissues

The values observed have *not* been corrected for dry weight fat content or chloride space They do not represent true intracellular content However it is of interest to compare these rapidly achieved results with those of the more detailed procedures as reported by different investigators for frog muscle The results are shown in Table III

Patients under Therapy with ACTH and/or Cortisone

It has long been appreciated that the clinical symptomatology associated with hypopotassemia as seen in its most extreme forms

Biopsy Technique

Procaine ½% is injected intracutaneously A two inch longitudinal incision is made in the prominent medial surface of the medial head of either gastrocnemius muscle Additional procaine is injected subcutaneously No procaine is injected into the muscle itself The muscle sheath is cleaned and reflected This is painless Cutting the muscle is painful but may be completed quickly by the use of scissors One gram of muscle is removed

Procedure

The muscle is placed within a 4′ x 4′ gauze which has been wetted with tap water and completely wrung out This retards evaporation *The muscle is blotted gently and then transferred to another* similar 4′ x 4 gauze where the blotting is repeated This suffices to remove most of the blood The specimen wrapped in the 4 x 4′ gauze is then brought to the balance A round cover slip is weighed and the muscle is divided by a scalpel blade into two parts one about 100 mgs for dry weight No single piece should exceed 0 3 gms in weight From 200 to 500 mgs are placed on the cover slip and weighed rapidly The cover slip and muscle are both placed within a large dry test tube calibrated at 25 ml The volume of the cover slip is small enough to be ignored We use urea digestion tubes A weighing bottle is now weighed and the remaining small piece of muscle is placed in it and weighed This is now placed in a drying oven at 110° C and dried to constant weight usually 24 hours

Two rapid procedures are available which agree with each other and with dry ashing

a One is *digestion with HNO_3* 0 5 cc concentrated HNO_3 is added to the test tube which is placed in a steam bath for one hour The tube is unstoppered At the end of the hour distilled water is added up to the 24 cc mark This is well mixed and filtered since there may be a small amount of residue which could clog the spray tip of the flame photometer The standards used contain sodium and potassium in the same approximate ratio as the unknown and a similar concentration of HNO_3 (2% by volume)

b The other method employs no chemicals and is a modification of the method of Stone and Shapiro[1] About 24 cc of distilled water are added to the muscle in the test tube This is placed in the steam bath for one hour The tube is unstoppered Water is added to the 25 cc mark and the solution is well mixed and filtered The muscle at most 0 5 gm which is at least 50% water cannot affect volume by more than 1% which is neglected In this case the flame photometer

mately one half gram of sodium a day and a total of three and a half grams of potassium a day *

The fact that on this regimen there is no striking shift in muscle electrolytes before and after therapy is illustrated by Table IV

We have data under further study in this laboratory which show pronounced reciprocal changes in tissue sodium and potassium values

Table IV

SODIUM AND POTASSIUM DETERMINATIONS ON HUMAN MUSCLE IN PATIENTS UNDER ACTH AND CORTISONE THERAPY

Patient	Before Therapy		After Therapy	
	Sodium (mEq/kg of fresh tissue)	Potassium (mEq/kg of fresh tissue)	Sodium (mEq/kg of fresh tissue)	Potassium (mEq/kg of fresh tissue)
No 1	41	70	39	102
No 2	30	99	35	101
No 3	55	80	41	96

with this method Some of these values are given in Table V Because of changes in technique these preliminary results must be viewed with considerable caution

CONCLUSIONS

The multitude of factors which enter into the calculation of the true value of intracellular fluid are appreciated However what we are striving for at present is as follows

a To see if tissue sodium and potassium extravascular values fall within the same range for an average person

b To detect *gross* changes in tissue sodium and potassium levels

Further studies are in progress in this laboratory in an effort to expand the present data This method may then prove to be a good *clinical* tool which could be of value in the determination of the type of electrolyte therapy for patients

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* It is realized that this experimental regimen is not always adequate to handle potassium balance precisely in patients under therapy

Table II

SODIUM AND POTASSIUM DETERMINATIONS IN HUMAN MUSCLE

Muscle	Sodium (mEq /Kg of fresh tissue)	Potassium (mEq /Kg of fresh tissue)	Sodium + Potassium (mEq /Kg of fresh tissue)
Rectus	34	84	118
Sternocleidomastoid	37	91	128
Rectus	40	90	129
Rectus	37	84	122
Sternocleidomastoid	39	93	132
Rectus	46	94	140
* Psoas	35	93	128
Lumbar group	28	106	134
* Pectoralis	33	102	134
Back muscles	46	81	127
Rectus	40	90	130
Rectus	33	96	129
Rectus	38	87	124
AVERAGE	36	92	128
RANGE	28–46	81–106	118–140

Analyses done on post mortem tissue

in familial periodic paralysis and more recently as encountered clinically in instances of prolonged administration of ACTH and/or cortisone is not closely correlated with the plasma level. Indeed balance studies have shown that negative balance of potassium can occur over considerable periods without affecting plasma levels.

One of the first applications of the technique described above in our clinic has been the study of electrolyte levels in striated muscle on patients before and after ACTH and cortisone therapy whose dietary regimen has been aimed at maintaining proper balance of sodium and potassium, i.e. on a standard diet containing approxi

Table III

SODIUM AND POTASSIUM IN FROG MUSCLE

Investigator	Sodium (mEq /Kg of fresh tissue)	Potassium (mEq /Kg of fresh tissue)	Sodium + Potassium (mEq /Kg of fresh tissue)
Urano	14.2	73.0	87.2
Meigs	23.5	89.5	113.0
Katz	23.9	79.0	102.9
Fenn	25.4	83.0	108.4
AVERAGE	21.8	81.1	102.9
RANGE	14.2–25.4	73.0–89.5	87.2–113.0

tion which is being put into the flame photometer interference may occur with resultant deviations from actual values

I wonder if in studying tissues of individuals having large differences in sodium vs potassium content either compounded standards or an internal standard photometer was used

DR SHELDON MARGEN I would like to ask a question In your series did you also simultaneously perform dry weight analysis on fat extracted tissues? If you did how much variation did you get by that procedure?

I think comparing results obtained by different investigators and showing the variations between their results isn t quite fair It is obtained by the methods they used because they undoubtedly all used rather different methods for their determinations

DR DAVID RUTSTEIN The distribution of water and electrolytes will be determined in part by the effect of adrenal corticoids on cell membrane permeability Experiments were performed in our laboratory in an attempt to obtain data on the effect of steroids including cortisone on water exchange across cell membranes using the well established technique of measuring changes in size of eggs of certain marine animals particularly Arbacia and Chaetopterus The eggs are photographed under constant conditions and the area of the great circle of the eggs is measured by a planimeter Through this technique it was possible to measure the effects of steroid compounds including desoxycorticosterone cortisone estradiol testosterone and progesterone on the permeability of the cell membrane to water Although a number of these hormones caused striking changes no change whatsoever was noted in those eggs exposed to cortisone The eggs did remain viable throughout the course of this experiment as demonstrated by cleavage following fertilization with sperm

DR S HOWARD ARMSTRONG JR The most important and at the moment experimentally insoluble problem in the expression of analytical data on tissue is—Milligrams and/or milli equivalents per unit of *what?* Many expressions have been used for the what all arbitrary e g per unit of wet tissue weight per unit of dry tissue weight per unit of dry fat free tissue weight per unit of tissue ash weight per unit of total tissue protein nitrogen per unit of total tissue water Of course the last would be from the standpoint of thermodynamic equilibrium relations the most useful expression if there were any real way to decide without the use of a number of

Table V

SODIUM AND POTASSIUM CONTENTS OF DOG STERNOCLEIDOMASTOID MUSCLE AFTER RECEIVING I V 9% NACL RAPIDLY

	Time	Sodium (mEq/Kg of fresh tissue)	Potassium (mEq/Kg of fresh tissue)	Sodium + Potassium (mEq/Kg of fresh tissue)	H_2O Content
DOG No 1	Before infusion	53	123	176	72
19 kilograms					
3000 cc in 1 hr	10 min after completion	44	109	153	72
	7 hours later	57	79	136	79
DOG No 2	Before infusion	38	78	116	73
16 kilograms					
2500 cc in 1 hr	10 min after completion	52	57	109	76
	7 hours later	145	36	181	76
DOG No 3	Before infusion	53	79	132	75
(Right ventricle frozen 2 weeks previously)	10 min after completion	61	60	121	76
17 kilograms 2500 cc in 1 hr	7 hours later	114	46	160	76

2 Mudge G H and Vislocky K Electrolyte Changes in Human Striated Muscle in Acidosis and Alkalosis J Clin Invest 28 482 (May) 1949

DISCUSSION

DR JACK METCOFF I should like to make two comments about Dr Farago s paper—one in relation to the analyses of muscle in children—that is the possibility of having a reversal of sodium and potassium content or marked alteration in sodium and potassium content in children similar to that of atrophic muscle Antipyrine spaces or D_2O Spaces in children indicate a very small quantity of fat relative to the normal adult muscle Correction of the data for fat content and quantitation on the basis of fat free tissue might bring the results somewhat more in line with those of other observers

It may have been fortuitous that the tissues we examined con tained relatively similar quantities of sodium and potassium In the presence of large discrepancies in these two ions present in the solu

22

Metabolic and Clinical Effects of Corticosterone (Compound B) in Man

*Jerome W Conn Stefan S Fajans * Lawrence H Louis and Betty Johnson*

UNIVERSITY HOSPITAL AND UNIVERSITY OF MICHIGAN MEDICAL SCHOOL ANN ARBOR

The limited availability of Compound B has prevented thorough study of its clinical and metabolic effects in man In 1940 85 mg of natural Comp B became available to Thorn and 90 mg to Leob and their respective associates Thorn gave the whole 85 mg in one day to a case of Addison s Disease and reported a desoxycorticosterone like effect upon electrolyte metabolism and also definite effects upon carbohydrate and protein metabolism Leob s group spread its 90 mg over a 5 day period in an Addisonian and reported no significant effect upon either inorganic or organic metabolism During the ensuing 10 year period no further reports on Comp B in man have appeared

Through the courtesy of Dr H F Hailman of the Upjohn Company 10 grams of Crystalline Compound B in aqueous suspension was made available for our study Prolonged metabolic balance studies have been carried out upon 2 normal men and upon 2 cases of Addison s disease one with a coexisting diabetes mellitus Observations upon 2 more cases of Addison s disease have been made Each of the latter patients has received 25 mg of Compond B daily for 60 days A very rapid and remarkable return to normal health has occurred in the Addisonians receiving Compound B

Fig 1 demonstrates the effect of large doses of Compound B on renal excretion of electrolytes in a normal subject It is seen that at a dosage level of 100 mg per day there occurred sharp retention of sodium and chloride and that at 200 mg per day a similar but more intense response occurred It is significant that escape from sodium and chloride retention occurred at both dosage levels while the compound was still being administered This same phenomenon occurs

* Research Fellow in Medicine of the American College of Physicians 1949–1950

arbitrary assumptions how much tissue water in the specimen analyzed is inside and how much outside the cell membrane

Assumptions can be circumvented in the instance of the red cell by use of the centrifuge which gives reasonably complete separation of the cell from its fluid environment and in our laboratory in an extension of the studies for which initial values are here reported we are running red cell determinations simultaneously with muscle determinations

No such easy method exists for separating cellular elements and fluids in muscle tissue Thus whereas Schick Hass and Ashley in our Department of Pathology have developed very beautiful techniques for studying isolated myofibrils both enzymatically and mechanically separated from muscle and Ashley and coworkers have prepared for publication superb electron microscopic studies of the preparations and have shown contractility from adenosine triphosphate this work is useless from the standpoint of electrolyte studies because the method of separation completely alters the electrolyte distribution with respect to sodium and potassium (Calcium and magnesium have not yet been studied)

Thus we have used milli equivalents per kilogram of fresh tissue in expressing our results and in serial determinations have in addition simply done dry weight determinations to make sure that the total amount of water per unit of fresh tissue does not vary significantly because gross changes are what we are looking for

It is our impression from data that were not available at the time that this manuscript was prepared that whatever the long term effect of ACTH and cortisone is on sodium and potassium balance the initial effect does not take place at the level of the cell membrane in terms of redistribution of electrolytes within the first 24 hours within the limits of the method we have used

We have also recently picked up in certain clinical situations changes in muscle electrolyte distribution as gross as those reported in the last table for the muscle of dogs given infusions of NaCl The significance of these taken together with simultaneous blood levels in terms of equilibrium will need a good deal of time to work out

more intense Increased renal excretion of potassium is observed during administration of corticosterone

Fig 4 compares in this second normal subject the relative effects upon urinary electrolytes of corticosterone (200 mg /day) and ACTH (100 mg /day) Again ACTH produces the more intense effects

Fig 5 indicates the results obtained in both normal subjects with respect to renal excretion of organic elements under the influence of administered Compound B Subject G A showed no significant

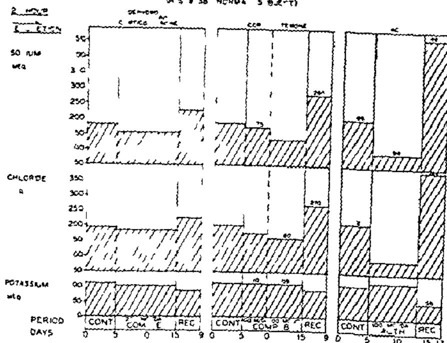


Fig 2

increases in urinary glucose uric acid or nitrogen Subject R S however demonstrated real increases in urinary excretion of glucose and uric acid but there was no effect upon urinary nitrogen

Fig 6 compares the effects upon glucose tolerance in Subject R S of Compound B Compound E and ACTH It will be recalled that this is the subject whose urinary glucose and uric acid increased on Compound B It is seen that ACTH produced the greatest impairment of glucose tolerance and that the effect of cortisone was greater in this respect than of corticosterone

Fig 7 shows that both normal subjects increased their urinary

in normal people under continued treatment with desoxycorticosterone It will be observed that changes in the hematocrit values and in body weight correlate well with the changes in renal excretion of sodium and chloride In this subject renal excretion of potassium was not affected significantly The blood pressure rose mildly in the Compound B period

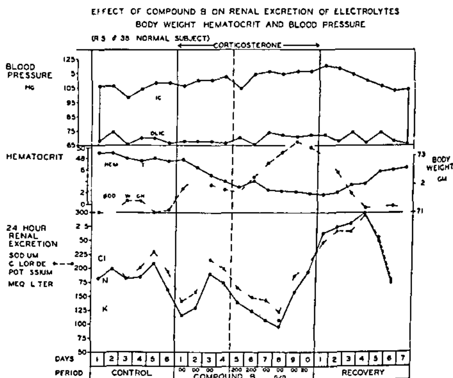


FIG 1

Fig 2 compares in this same normal subject the relative effects upon urinary electrolytes of cortisone (Compound E) Corticosterone (Compound B) and ACTH It is observed that at a dose level of 200 mg per day Compound B causes greater retention of sodium and chloride than does Compound E Similarly the sodium and chloride diuresis upon cessation of these compounds is greater after Compound B than after Compound E On the other hand 100 mg per day of ACTH produces much more marked effects on renal excretion of electrolytes than is observed with either Compound E or Compound B at the dose level of 200 mg per day

Fig 3 shows the results obtained upon the second normal subject They are similar to those seen in the first normal subject but

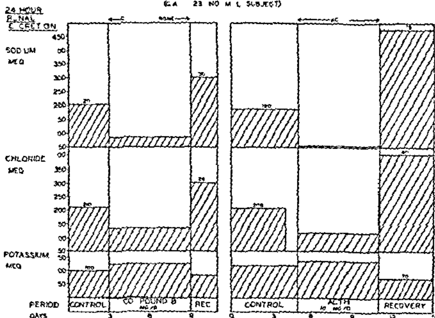
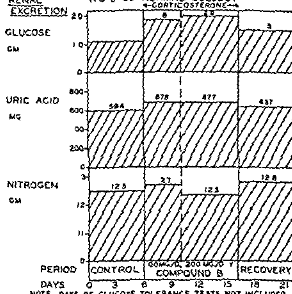

COMPARATIVE EFFECTS IN THE SAME PERSON OF COMPOUND B AND ACTH ON RENAL EXCRETION OF ELECTROLYTES
24 HOUR RENAL EXCRETION
SODIUM MEQ
CHLORIDE MEQ
POTASSIUM MEQ
PERIOD
DAYS
CONTROL
REC
CONTROL
ACTH
RECOVERY

Fig 4


EFFECT OF COMPOUND B ON RENAL EXCRETION OF GLUCOSE URIC ACID AND NITROGEN
24 HOUR RENAL EXCRETION
RS ♂ 38 NORMAL SUBJECT
←CORTICOSTERONE→
GLUCOSE GM
URIC ACID MG
NITROGEN GM
584
678
677
637
PERIOD
CONTROL
COMPOUND B
RECOVERY
DAYS
NOTE DAYS OF GLUCOSE TOLERANCE TESTS NOT INCLUDED

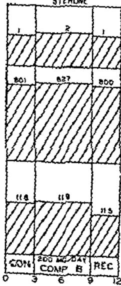

GA ♂ 23 NORMAL SUBJECT
←CORTICO STERONE
801
827
800
200 MG/DAY
CON
COMP B
REC

Fig 5

excretions of formaldegenic steroids while receiving large doses of Compound B Urinary 17 ketosteroids diminished mildly in Subject G A and remained unchanged in Subject R S

From these observations in the normal subjects one can conclude

1 That Compond B has pronounced effects upon electrolyte

EFFECT OF COMPOUND B ON RENAL EXCRETION
OF ELECTROLYTES HEMATOCRIT AND BODY WEIGHT
(G A ♂ NORMAL SUBJECT)

Fig 3

metabolism and that these effects are qualitatively similar to those produced by desoxycorticosterone

2 That Compound B excretes mild effects upon organic metabolism which are qualitatively similar to those produced by cortisone

3 That upon a milligram basis corticosterone has a more intense effect upon inorganic metabolism and a less intense effect upon organic metabolism than does cortisone

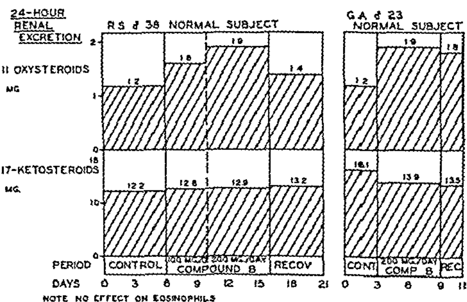

EFFECT OF COMPOUND B ON RENAL EXCRETION
OF 11-OXYSTEROIDS AND 17-KETOSTEROIDS
24-HOUR RENAL EXCRETION
R S ♂ 38 NORMAL SUBJECT
G A ♂ 23 NORMAL SUBJECT
11 OXYSTEROIDS
MG
17-KETOSTEROIDS
MG
PERIOD
CONTROL
100 MG/D
200 MG/DAY
COMPOUND B
RECOV
CONT
200 MG/DAY
COMP B
REC
DAYS
NOTE NO EFFECT ON EOSINOPHILS

Fig 7

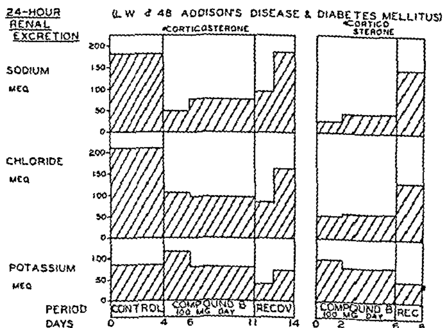

EFFECT OF COMPOUND B ON RENAL EXCRETION
OF ELECTROLYTES
24-HOUR RENAL EXCRETION
(L W ♂ 48 ADDISON'S DISEASE & DIABETES MELLITUS)
*CORTICOSTERONE
*CORTICO STERONE
SODIUM
MEQ
CHLORIDE
MEQ
POTASSIUM
MEQ
PERIOD
CONTROL
COMPOUND B 100 MG DAY
RECOV
REC
DAYS

Fig 8

Because we wished to demonstrate more critically the effects of Compound B upon organic metabolism a patient with coexisting Addison s disease and diabetes mellitus was studied next This patient was studied twice corticosterone having been administered in a dose of 100 mg per day Fig 8 shows the effects upon renal excretion of electrolytes Retention of sodium and chloride and diuresis

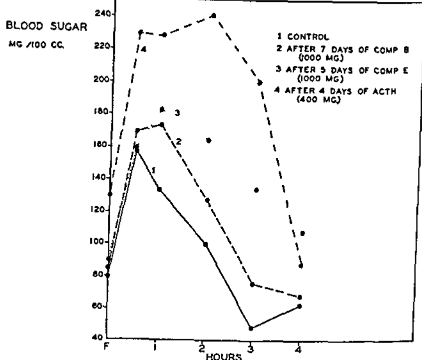


Fig 6

of potassium were more intense than in the normal subjects even when the latter received 200 mg per day of Compound B

Fig 9 demonstrates clearly the effects of corticosterone upon organic metabolism in this patient Significant increases were observed in urinary excretion of glucose and nitrogen as well as in the uric acid creatinine ratio In addition Fig 10 indicates that corticosterone exerts an anti insulin effect in confirmation of the early animal experiments of Jensen and Grattcn

In a set of experiments upon another case of Addison s Disease (Fig 11) we have compared the metabolic effects of 25 mg /day of

Table I

COMPARATIVE PHYSIOLOGICAL PROPERTIES OF DCA, COMPOUND E AND COMPOUND B

	DCA	*Cortisone (E)*	*Corticosterone (B)*
Electrolyte *Metabolism*	Intense	Mild and Variable	Good
Organic *Metabolism*	None	Intense	Mild
EEG	None	Good	Good
Pep	None	Good	Good
CI Absorption	Small	Good	Good*

* Preliminary

It is a property which we have all observed during administration of either ACTH or cortisone It is difficult to assign relative values with respect to a property as nebulous as this but corticosterone possesses it

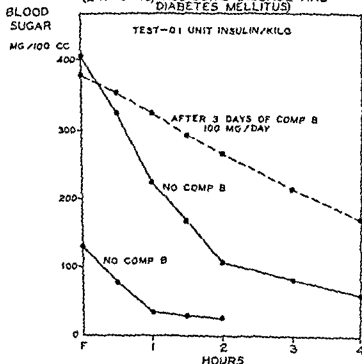


Fig 10

corticosterone with the effects of a mixture (aqueous suspension) of 20 mg /day of cortisone plus 2 mg /day of desoxycorticosterone It is observed that even with this small dose of Compound B a sharp desoxycorticosterone like effect is obtained in the Addisonian This effect is more intense than that observed with the cortisone desoxy corticosterone mixture Fig 12 shows that at this level of dosage neither Compound B nor the cortisone DCA mixture affected uri

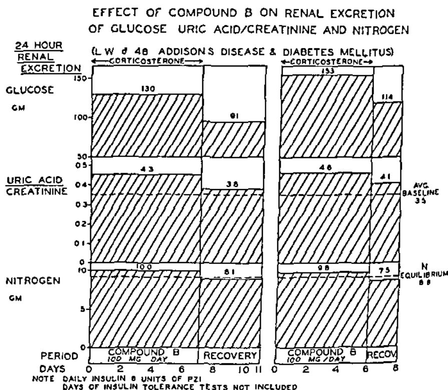


FIG 9

nary excretion of nitrogen In both experiments however the uric acid creatinine ratio rose to the same degree

Fig 13 indicates that the fasting hypoglycemia which is observed in some cases of Addison s disease can be prevented by the adminis tration of corticosterone

Table I is intended to portray a gross comparison of the physio logical properties of DCA cortisone and corticosterone as they relate to the untreated Addisonian A remark is required regarding the property which we have labelled as pep Although this is based wholly upon clinical observation it is nonetheless definite It consists of an intangible feeling of well being and of zest for activity and life

metabolism is much less intense than that of DCA giving the advantage of a wider safety range in dosage

2 Compound B also corrects the fasting hypoglycemia which many of these patients exhibit This effect upon organic metabolism although demonstrable in normals is much more evident in adrenal insufficiency

3 In doses as small as 25 mg /day Compound B imparts to the Addisonian a remarkable feeling of well being of pep and of zest for living In this respect it is similar to Compound E and both compounds improve the abnormal Addisonian electroencephalogram

4 Addisonians receiving cortisone alone are frequently improved when a small amount of DCA is added daily Compound B

PREVENTION OF FASTING HYPOGLYCEMIA WITH CORTICOSTERONE

(A H ♀ 38 ADDISON S DISEASE)

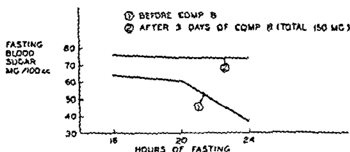


FIG 13

alone appears to produce the physiological and metabolic effects of this combination (cortisone + DCA)

5 Since DCA is poorly absorbed from the gastrointestinal tract of man Compound B may prove to be the steroid of choice for adequate oral substitution therapy in Addison s disease

6 Finally it appears that the presence of an oxygen atom on carbon 11 imparts metabolic activities which are responsible for producing the feeling of well being This particular activity does not parallel any of the metabolic changes which we have been able to measure

DISCUSSION

DR W D ROBINSON (Ann Arbor Michigan) We have had the opportunity to study the anti rheumatic effect of corticosterone (Compound B) in one patient

COMPARATIVE EFFECTS OF COMP B AND OF COMP E + DCA (MIXED)
UPON RENAL EXCRETION OF ELECTROLYTES

(F H ♂ 43 ADDISONS DISEASE)

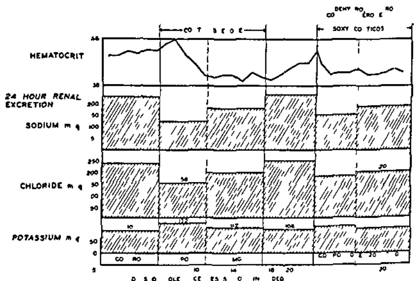


Fig 11

COMPARATIVE EFFECTS OF COMP B AND OF COMP E + DCA (MIXED)
UPON URINARY NITROGEN AND URIC ACID-CREATININE RATIO

(F H ♂ 43 ADDISONS DISEASE)

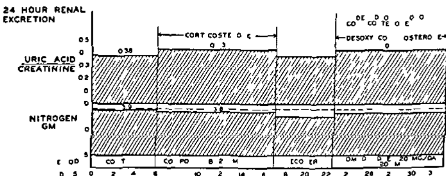


Fig 12

CONCLUSIONS RE GENERAL PROPERTIES IN NORMALS

1 When administered in untreated Addison's disease Compound B corrects the disturbance of electrolyte metabolism Milligram for milligram the effect of Compound B upon electrolyte

metabolism is much less intense than that of DCA giving the ad vantage of a wider safety range in dosage

2 Compound B also corrects the fasting hypoglycemia which many of these patients exhibit This effect upon organic metabolism *although demonstrable in normals is much more evident in adrenal insufficiency*

3 In doses as small as 25 mg /day Compound B imparts to the Addisonian a remarkable feeling of well being of pep and of zest for living In this respect it is similar to Compound E and both compounds improve the abnormal Addisonian electroencephalogram

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PREVENTION OF FASTING HYPOGLYCEMIA WITH CORTICOSTERONE

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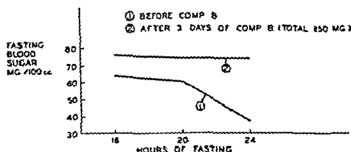


FIG 13

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DISCUSSION

DR W D ROBINSON (Ann Arbor Michigan) We have had the oppor tunity to study the anti rheumatic effect of corticosterone (Com pound B) in one patient

This patient (Fig 14) with typical rheumatoid arthritis was given intramuscularly 200 mg of Compound B a day for a period of ten days During this time there was slight subjective improvement on the fourth day which was not progressive There was no objective improvement at this dosage level no consistent effect on the sedimentation rate and no relapse when corticosterone was discontinued

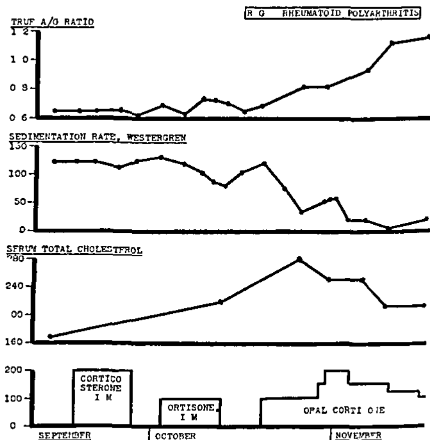


Fig 14

After an interval of five days 100 mg per day of cortisone was given intramuscularly for ten days At this dosage level there was a definite subjective effect on the third day progressive subjective and objective improvement was noted with a definite decrease in the sedimentation rate relapse occurred after cortisone was discontinued The patient subsequently responded very nicely to oral cortisone

It appears from this study that corticosterone does not have a high

degree of anti rheumatic activity Further studies will be necessary to determine whether it is devoid of such an effect

DR PETER H FORSHAM These beautiful studies of Dr Conn s remind one of work done by various groups on what is known as Compound A (dehydrocorticosterone) differing from corticosterone by the fact that there is an O instead of OH on carbon 11

Compound A seemed to show most of the potentialities of corti sone in Addison s disease but did not have the same effectiveness that one would generally find with cortisone

Based upon these investigations done in 1946 some of which were frankly negative while others showed all that we saw tonight it was decided those who had to make the decision not to make any more Compound A but to try to make a small batch of cortisone Out of that grew the eventual discovery of the use of cortisone in rheumatoid arthritis

One other interesting thing was that although the metabolic changes were always of the borderline type with dosages from 50 to 100 mgs a day the improvement in the health of the twelve patients with Addison s disease whom we carried on Compound A for three months or more was universally present

We concluded that Compound A was really quite valuable in Addison s disease and would have continued to press for its manu facture for Addison s disease had it not been for the fact that the usefulness of cortisone in another much more widespread disease made this a practical agent of choice in the treatment of Addison s disease

The point brought out about the fact that corticosterone actually leads to retention of quite a bit more sodium than cortisone does is a very important one If we treat patients with Addison s disease with only 12 5 to 25 mg of cortisone per day they get back to perfect health provided one adds on one or two mg of desoxycorticosterone or else 10 gm of salt daily to preserve sodium balance With corticos terone that would not seem to be necessary and that makes it a pref erable compound

I have a brief question What happened to the eosinophils and the lymphocytes? With Compound A they were not affected with cortisone depressed

DR JEROME W CONN We observed no significant fall of the eo sinophils with Compound B We did not study the lymphocytes While the eosinophils did not fall in these experiments it is possible that with oral corticosterone they may act differently from what they do when one gives the compound parenterally

That brings up a point that I did not mention and one that I believe may be important Preliminary investigations indicate that corticosterone is probably well absorbed by mouth If so, then corticosterone which combines the activities of cortisone and desoxycorticosterone in the Addisonian will have a distinct advantage Cortisone is effective by mouth but desoxycorticosterone is not well absorbed by the gastrointestinal tract Thus the single compound corticosterone may make available good complete oral substitution therapy

PART III

NITROGEN METABOLISM

23

Comparative Effects of ACTH and Stress in Nitrogen Metabolism*

Frank L Engel

DUKE UNIVERSITY DURHAM

For some time the concept has been widely accepted that the negative nitrogen balance which follows stress in man and animals is a direct consequence of the enhanced secretion of ACTH and adrenal cortical hormones characteristic of that state Since the original demonstration by Long Katzin and Fry of a gluconeogenetic effect of certain adrenal steroids[1] the adrenal cortex has generally been considered to have a direct influence on the stimulation of protein catabolism

Recently there has been increasing scepticism regarding the above concept of the relation between the adrenal and stress The first clear-cut evidence against it came in the report of Ingle et al[2] who reported that cortin maintained adrenalectomized animals responded normally with regard to nitrogen metabolism after leg fractures Similar observations had been reported earlier by Selye and Dosne[3] concerning the blood sugar alterations in response to traumatic shock in cortin maintained adrenalectomized rats From such studies Ingle[4] has proposed the basic concept that the adrenal cortex is necessary but not responsible for the change in nitrogen metabolism following stress The present communication gives further evidence that the effects of stress cannot be simply the result of enhanced adrenal cortical secretion It will be shown that the increase in nitrogen metabolism following non specific stress occurs more rapidly in

* This study was aided by a grant from the American Cancer Society Administered by the Committee on Growth of the National Research Council

animals with intact adrenal glands than it does in similar animals after even massive doses of ACTH intravenously

The technique used was that of the measurement of the rate of urea formation in the bilaterally nephrectomized rat a highly sensitive method for measuring the time relationships in nitrogen metabolism in response to various stimuli[5,6] In these experiments rates of urea formation were measured at 3 hour intervals in rats nephrectomized 18 hours before The results are expressed as milligrams of urea nitrogen formed per 100 grams body weight per hour

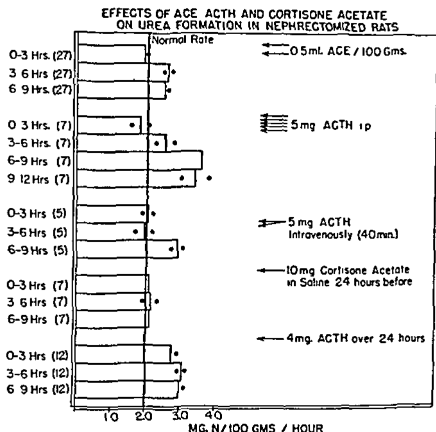


Fig 1

Figure 1 illustrates the effects of adrenal cortex extract (Upjohn) ACTH (Armour) and Cortisone Acetate (Merck) on urea formation Five mg of ACTH when injected intraperitoneally either as a single dose (not shown in figure) or in divided doses at 30 minute intervals or when infused intravenously over 40 minutes had no significant effect on urea formation until 3–6 hours after the beginning of the injection A similar delay occurred after the injection of A C E in

dicating that the time for the stimulation of the adrenal cortex by ACTH was not the limiting factor in the metabolic response One mg of ACTH injected subcutaneously at 6 hour intervals for 24 hours induced a sustained increase in nitrogen metabolism whereas 10 mg of the crystalline suspension of Cortisone Acetate had no measurable effect in 24 hours The same dose injected 48 hours be

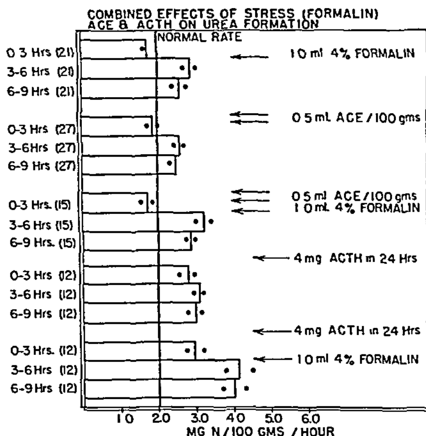


Fig 2

fore however does produce a significant increase in nitrogen metabolism

In contrast non specific stress (formalin subcutaneously epinephrine and marked insulin hypoglycemia) stimulated an increase in nitrogen metabolism detectable within the first 3 hours after injection The magnitude of the response was not modified by doses of ACTH or A C E which would most certainly suppress further endogenous secretion of ACTH and adrenal steroids (Figures 2 and

3) The more prompt response to stress than to ACTH indicates that enhanced secretion of adrenal hormone cannot be responsible for the stress reaction while the failure of even massive doses of ACTH to modify the stress implies that the level of secretion of adrenal hormone is not predominant in determining the magnitude of the stress response in nitrogen metabolism

The necessity for adrenal hormone to make possible the initiation and the sustaining of the response to stress is shown in Figure 3 in the case of epinephrine stress A large dose of epinephrine (0 1 mg

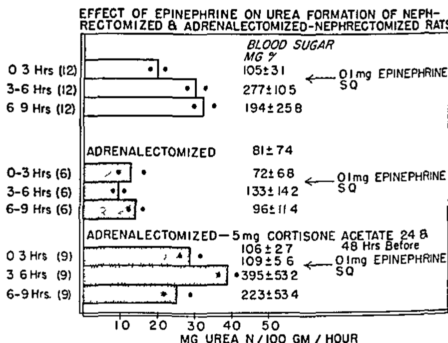


FIG 3

subcutaneously) served as a brisk stimulus to urea formation in animals with intact adrenals but not in DOCA maintained adrenalectomized rats However when given to adrenalectomized rats maintained with Cortisone Acetate for 48 hours a prompt but temporary increase in urea formation occurred confirming the earlier observations of Ingle with regard to fractures [2] The failure of this response to be sustained in this preparation is believed to be due to the fact that the supply of hormone from the cortisone depot was insufficient to maintain the response in contrast to the normal animal which could increase its adrenal secretion as needed

When milder stresses (0 5 ml formalin small doses of insulin)

were used no immediate increase in urea formation occurred However if the animals were treated with A C E cortisone or ACTH an immediate acceleration of nitrogen metabolism comparable to more severe stresses took place (Figure 4)

It would thus appear that provided adequate amounts of adrenal hormone are available non specific stress by an unknown mechanism induces a change in nitrogen metabolism the maintenance of which is dependent on a continuing supply of adrenal hormone

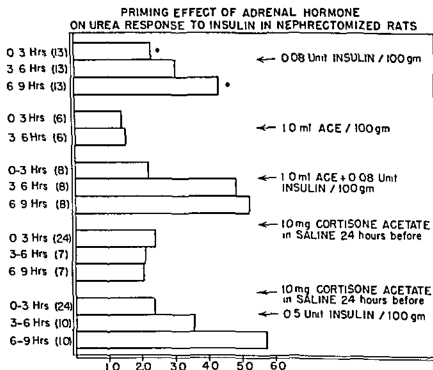


Fig 4

Conversely in the presence of excess adrenal hormone mildly stressful situations which in themselves are not severe enough to produce measurable changes in nitrogen metabolism now become associated with such changes This suggests that excess adrenal hormone sensitizes the organism to respond more readily to minor changes in its internal or external environment by increasing protein catabolism

As shown elsewhere[6 7 8 9] the protein catabolic response to either adrenal hormone or stress in the fasted animal can be largely or completely abolished by administration of carbohydrate or amino acids The spontaneous increase in appetite seen in patients with Cushing s

Syndrome or receiving ACTH or cortisone may thus represent a homeostatic response to protect the organism which is hypersensitive to stress against undue loss of nitrogen

If one accepts the view that the well known metabolic response to stress in the healthy organism reflects a reaction designed to protect the organism from damage then the role of the adrenal cortex in sensitizing the body to react in this fashion becomes more meaningful Extension of this concept to the other metabolic reactions generally identified with hyperadrenal corticism and stress and to the multitude of other types of defense reactions which are linked with the adrenal cortex would give a basis for the understanding of the therapeutic significance of and the toxic reactions to ACTH and cortisone

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DISCUSSION

DR HANS SELYE I would like to add just one or two words to Dr Engel s presentation to underline its importance and scope

Not only as far as nitrogen metabolism is concerned but for instance as far as involution of lymphatic organs is concerned the same mechanism seems to apply Adrenalectomized animals maintained on salt do not show thymico lymphatic involution upon exposure to stress (e g formaldehyde injections) However if you administer a sub threshold amount of adrenal cortical extract the

subsequent injection of formaldehyde will cause a very marked involution

Apparently here the cortical extract has sensitized the animal to the non specific effect of the formaldehyde

I think one of the most important implications of this sensitization phenomenon might be in the so-called collagen diseases or what we like to consider diseases of adaptation You will recall that mineralo-corticoids such as desoxycorticosterone acetate failed to cause any hypertension or visible vascular changes in the absence of dietary sodium On a normal sodium intake they do cause such changes On excess sodium they cause such changes even at very low dosage levels

Perhaps the lack of evidence for any marked increase in the urinary excretion of corticoids during clinical syndromes resembling those experimentally caused by DCA may find its explanation in this phenomenon of sensitization Obviously even normal amounts of mineralo-corticoids could be conducive to disease if their effect were greatly augmented by sensitizing metabolic changes

DR FRANK L ENGEL I am glad Dr Selye brought up the point about lymphoid tissue because I think it is extremely important I wonder whether it might not be equally true with respect to the eosinophil and whether we are thinking too much in terms of the eosinophil fall purely as an effect of adrenal hormone action rather than as a conditioned response

I think the case that Dr Wolfson mentioned this morning of a patient with a fairly steady eosinophil count while on ACTH who then got renal colic and had the eosinophil count fall promptly may possibly be an example of a similar phenomenon

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Some Clinical and Metabolic Responses of Premature Infants to ACTH Including Its Effect on Aromatic Amino Acid Metabolism*

S Z Levine, Henry L Barnett, C Warren Bierman† and Helen McNamara

THE NEW YORK HOSPITAL–CORNELL MEDICAL CENTER AND CORNELL UNIVERSITY MEDICAL COLLEGE NEW YORK

Most premature infants receiving high protein ascorbic acid free diets show a *specific defect in tyrosine and phenylalanine me*tabolism manifested by urinary excretion of the tyrosyl derivatives p hydroxyphenylpyruvic acid and l p hydroxyphenyllactic acid Without exception administration of ascorbic acid promptly abolishes the defect An interrelationship between ascorbic acid and adrenocortical function has long been recognized Ascorbic acid present in the adrenal cortex in higher concentration than in other *tissues decreases following ACTH administration or exposure to* stress The interrelationships between the p[illegible] and adrenal glands and vitamin C prompted this study of th[illegible] AC[illegible] tyrosyluria and coincidentally on body weight c[illegible] circulating eosinophiles of prematu[illegible]

MATERIALS

The study group comprised [illegible] 1112 to 2240 grams at birth [illegible] diet of 6 grams of protein 120 gram body weight Hormone [illegible] were between 11 and 58 days grams in weight

Figure 1 shows the number of hormone therapy the range ment Seventeen infants recei[illegible]

* This investigation was aided by a [illegible]
† Supported by a Fellowship from Th[illegible]

doses given in divided amounts every 6 hours varied between 12 5 and 100 mg and the total course between 12 5 and 500 mg over 1 to 10 day periods Fifteen infants received one or more of the six steroids listed in the table as follows eight observations on six in fants with cortisone (compound E) two infants desoxycorticosterone acetate two infants testosterone propionate three infants progester one one infant each dehydrocorticosterone (compound A) and Reichstein s compound L Casual specimens of urine were ana lyzed daily for tyrosyl derivatives

	PTS	OBS	DOSAGE - MG		
	NO		PER DAY RANGE	DAYS NO	TOTAL RANGE
ACTH	17	24	12 5-100	1-10	12 5-500
E	6	8	50-100	3 5-10	280-760
DCA	2	2	2-3	5-6	12 15
TESTOSTERONE	2	2	5-10	7	35 70
PROGESTERONE	3	3	25-100	7	175-700
A	1	1	100	7	700
L	1	1	100	7	700

Fig 1

OBSERVATIONS ON THE METABOLIC DEFECT

The metabolic aberration was corrected in all of 19 observations on 17 premature infants male and female white and colored who received ACTH therapy for three or more days in a total dosage exceeding 60 mg Figure 2 shows the responses in 8 infants who re ceived from 12 5 to 25 mg of ACTH daily Tyrosyl compounds consistently disappeared from the urine of all infants as early as the fifth day of hormone therapy and always by the second day of the post period This delayed response contrasts with the more rapid action of ascorbic acid With this vitamin in adequate dosage the response occurs within 24 to 48 hours of therapy

Since abolition of the defect with ACTH might reasonably be assumed to be mediated through stimulation of adrenocortical ster oids a number of such steroids were investigated Desoxycorticoste rone dehydrocorticosterone Reichstein s compound L progester one and testosterone were ineffective in the dosages employed Results with cortisone remain inconclusive Correction of the meta

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MATERIALS AND METHODS

The study group comprised 26 premature infants weighing from 1112 to 2240 grams at birth maintained on a constant vitamin C free diet of 6 grams of protein 120 calories and 150 cc of fluid per kilogram body weight Hormone therapy was started when the infants were between 11 and 58 days of age and between 1320 and 2500 grams in weight

Figure 1 shows the number of infants and observations the types of hormone therapy the range of dosage and the duration of treatment Seventeen infants received ACTH in 24 observations Daily

* This investigation was aided by a grant from the Playtex Park Research Institute

† Supported by a Fellowship from The National Foundation for Infantile Paralysis

day and in all infants by the sixth day of treatment Even more striking were the ravenous appetites which became so marked that by the sixth day of therapy 16 of the 17 infants were continuously hungry Overactivity and crying decreased rapidly and appetites re turned gradually to normal pre treatment levels within the first 4 to 6 days of the post periods

The effect of the adrenal steroids on these clinical features was negligible by contrast Cortisone therapy produced slight increase in activity appetite and crying in 3 of 6 infants while the other steroids produced no deviation from pre treatment behavior pat terns

EFFECT ON EOSINOPHILES

A fall of 50 per cent or more in circulating eosinophiles occurred in only 3 of 19 observations four hours after an initial dose of 3 125 to 12 5 mg of ACTH A depression of this magnitude was however present in all infants by the end of 24 hours of divided therapy After 48 hours of treatment circulating eosinophiles virtually disappeared from the peripheral blood Return to pre treatment levels following discontinuation of therapy was gradual only one half of the infants reaching or exceeding these values by 48 to 72 hours of the post periods

GENERAL DISCUSSION AND SUMMARY

ACTH given in adequate dosage over a sufficient period of time consistently abolished the defect in aromatic amino acid metabolism manifested by premature infants receiving ascorbic acid free high protein diets The mechanism of action of ACTH is at present ob scure It differs from that of ascorbic acid in that it requires more pro longed administration Since there was no change in plasma or white cell ascorbic acid levels and no increase in urinary ascorbic acid excretion in 2 infants during therapy the correction would not seem to involve the mobilization of stored ascorbic acid The failure of cortisone to correct the defect in five of eight observations in spite of high dosage needs further investigation

ACTH has a marked effect on the circulating eosinophiles clini cal behavior appetite and body weight of premature infants while the other steroids in the dosages used have variable or slight effects on these functions

DISCUSSION

DR WILLIAM A SILVERMAN In an effort to find a dose of ACTH that would inhibit the retinal changes in the early stages of retrolental

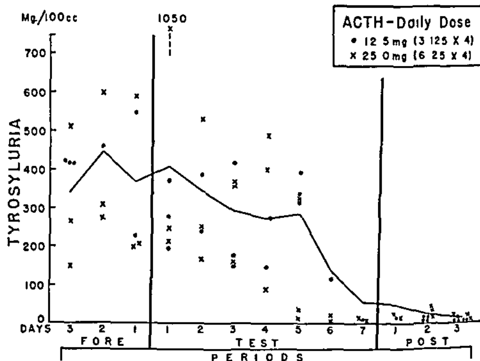


Fig 2 Effect of ACTH on tyrosyluria of premature infants

bolic defect was accomplished in 3 of the 8 observations Total dosages of at least 350 mg were required for correction and even these relatively large amounts were ineffective in 2 infants

EFFECT ON BODY WEIGHT

The effect of ACTH on the pattern of body weight changes was identical for all premature infants in all observations and at all levels of dosage The pattern may be described as follows

1 Substantial weight gains averaging 27 grams per day in pre periods of 1 to 2 weeks

2 Marked reductions in the rate of weight gain in test periods of one week with an average weight loss of 5 grams per day

3 Markedly accelerated weight gains averaging 51 grams per day in post periods of 1 to 2 weeks following cessation of ACTH therapy

The average weight gains for the combined test and post periods however closely approximated those of the fore period

CLINICAL BEHAVIOR

ACTH therapy produced a striking change in the behavior of infants Hyperactivity and a marked increase in volume vigor and duration of crying became evident in some by the end of the second

rebound rapid increase in weight averaged 2 weeks Following this the pre treatment rate was resumed

Serial measurements of the occipito frontal circumference of the head before during and after ACTH revealed changes in rate of growth that occurred concomitantly with the weight changes (Figure 4) Measurements of the length of the fibula were made on 6 infants

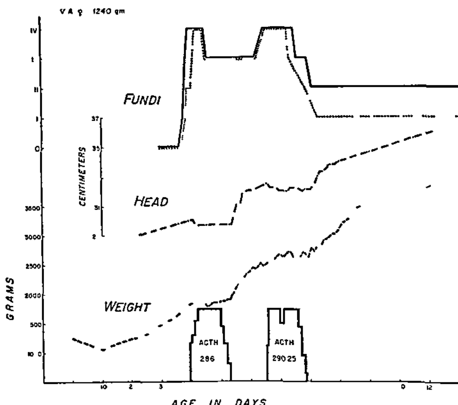


Fig 4 Effect of ACTH on fundi head growth and weight gain in premature infants

Serial roentgenograms of the fibula were made at a constant tube distance The length of the fibula was expressed as an average of the diagonals of this rectangular bone The dose of ACTH used had an inhibiting effect upon the growth in length of the fibula as can be seen in figure 5 A sharp rebound increase in length of the fibula occurred following cessation of ACTH in all but the one infant who received the greatest amount of ACTH (730 mgms in 20 days)

One experience with a smaller dose of ACTH and two experi

fibroplasia Doctors Richard L Day Frederick C Blodi and I have had an opportunity to observe the effects of ACTH administered to premature infants at the Babies Hospital

Moon and Evans Simpson and Li have described the inhibiting effect of adrenocorticotrophic extracts upon the growth of immature rats Becks Simpson Li and Evans and Simpson Asling and Evans have noted retardation of osteogenesis and chondrogenesis in immature rats Jackson has shown that when immature rats are refed

INHIBITING EFFECT OF ACTH ON WEIGHT GAIN

Pt	Birth Wt Grams	Wt Onset Rx Grams	Total ACTH Mgms	Dur Rx Days	AVERAGE WEIGHT GAIN GRAMS/DAY Before	During	Aft r
E S	1120	2160	182 75	14	18 7	21 5 *	49 2
M R	920	2620	359	20	31 5	7 8	55 8
L W	1330	3700	730	20		11 6	13 2 ***
V	1240	1850	286	14	34 2	4 2	53 6
(s nd cou se)		2520	290 25	14		13 1	50 0
C C	1160	2030	180	12	29 0	23 0	
K	850	1500	240	14	19 2	3 6	30 0
F	1360	2810	262 25	12	24 5	-23 6 **	44 5
J F	1450	2370	306	15		- 2 9	29 0
T B	1500	2220	209 25	10	28 2	18 9	42 5
A T	1340	1790	250	14	30 8	17 7	30 0
B	1775	2080	284	18	32 4	24 1 *	50 0
			A g G ins		28 1	10 1	39 5

* ACTH gi in tape i g do pri ipal g i du i g l tt r peri d
** Di rrh beginni g o ighth day f th py
*** Di rrh immedi t ly ft ti of th py

Fig 3 Inhibiting effect of ACTH on weight gain

after a prolonged period of food restriction abnormally rapid increase in weight occurs

Twelve prematurely born infants whose ages at the onset of therapy ranged from 28 to 84 days were given 8 to 40 mgms of ACTH per day for periods of 10 to 20 days (approximately 5 to 10 mgms per kilo per day) Growth inhibition was noted while receiving these doses of ACTH and marked acceleration of growth occurred after cessation of therapy In Figure 3 it can be seen that the average weight gain per day before the onset of treatment was 28 1 grams per day 10 1 grams per day while receiving ACTH and 39 5 grams per day following withdrawal of ACTH The duration of the

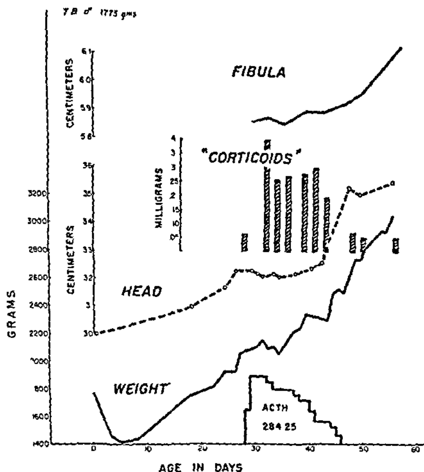


FIG 6 Effect of ACTH on growth of premature infants as reflected by fibular length head size and weight The relatively high urinary excretion of corticoids during ACTH administration should be noted

posterior pituitary hormones known to be present in ACTH The severity of the reactions appeared to vary with different lots of ACTH

ences with tapering doses of ACTH in which increased growth became evident as the dose was lowered (Figure 6) suggests that maximal adrenocortical stimulation may be necessary to produce growth retardation

DR JONATHAN T LANMAN (Bellevue Hospital and New York University College of Medicine New York City) The use of ACTH in the therapy of retrolental fibroplasia is being studied Signs suggestive of the disease were observed in twins thought to be identical In one some regression in signs was observed following therapy with ACTH in a dose of 20 mgm/day Subsequent exacerbation coinci

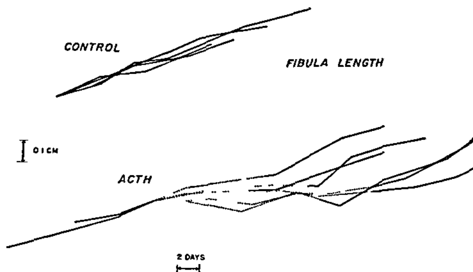


FIG 5 Effect of ACTH on bone growth as reflected by fibular length in premature infants

dent with lower dosage was observed During a second course of therapy with higher dosage the disease continued to progress The spontaneous fluctuations in the course of retrolental fibroplasia make hazardous the interpretation of the temporary regression observed in one twin

The second twin showed continuous progression of the disease with ACTH administered in doses of 20 mgm per day

ACTH therapy was associated with an arrest in weight gain irritability and increased activity and an increased urinary formaldehydogenic steroid output Administration of ACTH made from pork pituitaries was associated with immediate reactions characterized by localized blanching and generalized mottling and bluish discoloration of the skin and may have resulted from the small amount of

spite the increasing loss of total nitrogen the amino acid nitrogen excretion stabilized by the third treatment day and began to decline by the tenth day Following discontinuation of the drug the amino acid nitrogen quickly fell to pre treatment levels A spontaneous anabolic phase of nitrogen metabolism resulted which lasted for a month During the catabolic phase approximately 124 gms of nitrogen were lost and during the spontaneous anabolic phase 120 gms of nitrogen were retained The course of the amino acid nitro

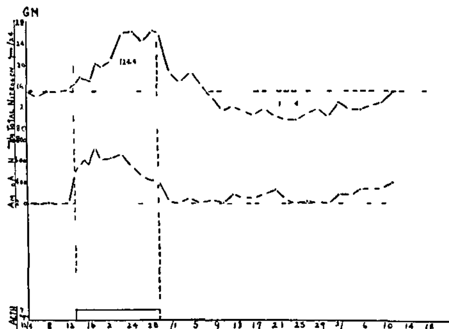


Fig 1

gen excretion during the anabolic phase was somewhat irregular with a tendency to rise near the end of the period

This pattern of amino acid nitrogen excretion during ACTH ad ministration has been observed in six subjects

In Fig 2 is shown the course of the fasting blood amino acid ni trogen levels in normal subject D K who was given 100 mgms ACTH H7911 daily for five days

There was an elevation of the fasting blood amino acid levels which persisted at least three days after the drug was stopped and associated with the increased loss in the urine of total and amino acid nitrogen The rapid drop in urinary amino acid nitrogen to pre treat ment levels is to be noted in contrast to the slower decline of the total nitrogen

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Effects of ACTH and Related Hormones on Amino Acid Metabolism*†

D M Bergenstal,‡ R L Landau, Joseph Kirsner and K Lugibihl

DEPARTMENT OF MEDICINE OF THE UNIVERSITY OF CHICAGO CHICAGO

This report is concerned with the study of the metabolism of amino acids during controlled anabolic and catabolic states This forms a part of a larger study of the endocrine regulation of growth processes in man

Holbrook et al [1] have shown a sharp increase in the excretion of certain amino acids in man during ACTH administration We have determined the urinary excretion of amino acid nitrogen and fasting blood amino acid levels in human subjects on metabolic balance studies In these subjects we have induced anabolic states of nitrogen metabolism with testosterone and catabolic states with ACTH (Armour) and an anterior pituitary preparation containing thyrotrophin

Subjects were placed on the metabolism ward and given an adequate constant diet and permitted their usual normal activity The urinary alpha amino acid nitrogen was determined by the copper method described by Albanese and Irby [2] The blood amino acid nitrogen was determined by the colorimetric method described by Russell [3]

RESULTS AND DISCUSSION

A normal 20 year college student G M was given 70 mgm ACTH H7911 daily for 17 days The dietary nitrogen was 17 5 gm /24 hours The general course of reaction to the ACTH is seen in Fig 1 There was a sharp rise in urinary nitrogen excretion which at the peak was 10 grams above pre treatment levels This was accompanied by an acute rise in amino acid nitrogen excretion De

* This work was supported in part by a grant from the American Cancer Society on recommendation by the Committee on Growth (A T Kenyon)

† We would like to thank Dr John R Mote and the Armour Laboratories for the ACTH and the pituitary preparation used in this study

‡ Damon Runyon Research Fellow in Medicine 1949–50

pituitary preparation rich in thyrotrophin This effect was characterized by a sharp catabolic response and an increase in amino acid nitrogen excretion The BMRs rose from −17 to +18 on the seventh day of treatment and this was followed by a gradual return to normal The amounts of plasma bound radioactive iodine measured after administration of radioactive iodine was significantly increased

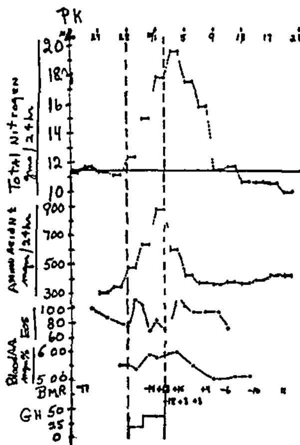


Fig 3

during therapy * The curve representing the amino acid nitrogen excretion seems to parallel the total nitrogen excretion This contrasts somewhat the ACTH experiments In the latter the urinary amino acid nitrogen rose more quickly to the maximum than total nitrogen and fell more promptly to the initial baseline on discontinuance of treatment

* We would like to thank Dr D W Clark and his colleagues for these determinations

The difference in fasting blood levels of amino acid nitrogen before and during ACTH administration probably does not reflect the maximum changes that take place during the day In one further experiment in which the blood and urine amino acid nitrogen was followed periodically throughout a control day and throughout a day during which ACTH was administered greater elevations in blood amino acid nitrogen were observed as compared with the control period following the noon and evening meals than had been apparent in the fasting states The fasting amino acid nitrogen level at

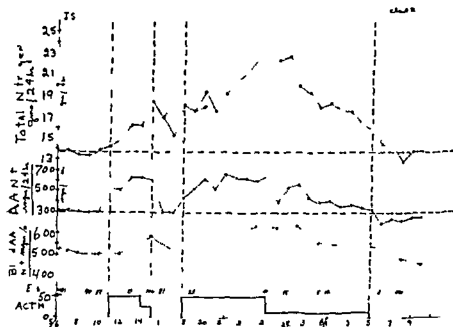


Fig 2

the end of the 24 hour period was 20% higher during ACTH administration This is a far greater alteration than can be accomplished by large changes in the protein of the diet Thus the fact that the fasting blood levels are elevated during ACTH administration would seem to indicate that a definite alteration in the metabolism of the amino acids has resulted

An alteration in the renal threshold may well occur associated with ACTH administration and may contribute to the loss of urinary amino acid nitrogen Elevations in the blood levels of amino acid nitrogen however indicate that more is happening than an increase in renal excretion

Fig 3 illustrates the effect of the administration of an anterior

Figure 5 shows the effect of ACTH on a eunuchoid subject On very low dose levels of ACTH H7911 an intense catabolic response and enhancement of amino acid nitrogen excretion was observed Alterations of as little as four mgms in ACTH dosage resulted in significant changes in nitrogen excretion Following discontinuation of the drug there resulted a sharp spontaneous anabolic phase that was associated with a brief retention of urinary amino acid nitrogen This nitrogen retention was well developed even in the absence of endogenous androgene A somewhat slower anabolic response of equal magnitude as shown in first portion of Fig 5 was set into



Fig 5

effect by testosterone This was not associated with amino acid retention

In a study on J S Fig 6 an anabolic state was induced with testosterone and this was associated with no significant alterations in fasting blood levels or urinary excretion of amino acid nitrogen

The mechanisms for the observed alterations in amino acid metabolism during induced catabolic states is not clear A study of possible alterations in the re absorptive capacity of the renal tubules is being undertaken

SUMMARY

1 During catabolic states of nitrogen metabolism induced by ACTH there is a sharp increase in amino acid nitrogen excretion

There was no evidence of participation of the adrenal cortex in this catabolic reaction as judged by the stability of the eosinophiles and of the 17 ketosteroids

The effect of ACTH however, on amino acid nitrogen excretion in a case in which increased amounts of thyroid hormone cannot contribute is shown in Fig 4 Subject J S had myxedema adequately treated with two grains of thyroid extract daily He had previously

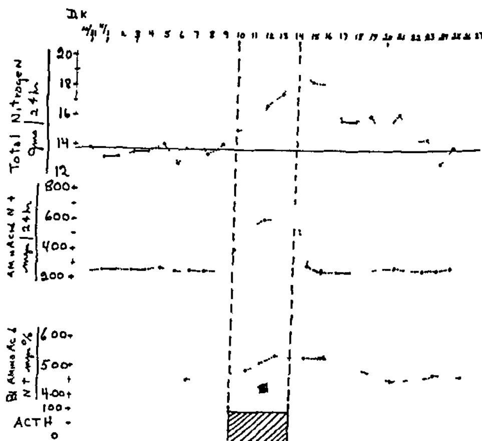


Fig 4

been given an anterior pituitary preparation rich in thyrotrophin without significant alteration in nitrogen excretion amino acid excretion or increases in BMR With ACTH H7911 administration the same patterns of response of the fasting blood and urine amino acid nitrogen excretion as seen in normal subjects was observed When the dose of ACTH was lowered to 12 5 mgm daily the eosinophiles remained depressed but the amino acid nitrogen excretion gradually returned to pre treatment levels There were no consistent alterations in BMR

3 Frame E G Russell J A and Wilhelmi A E The Colorimetric Estimation of Amino Nitrogen in Blood J Biol Chem 1943 149 255

DISCUSSION

DR F HOMBERGER At a recent ACTH Conference of the American Cancer Society in New York we reported preliminary observations indicating that the increased nitrogen excretion and the increased urine nitrogen/phosphorus ratio caused by ACTH were partially reversed by the oral administration of 5 mg per kg body weight of aureomycin This antibiotic was given because it has growth promoting properties in chickens and hogs and it seemed reasonable to expect that it might counteract the protein catabolic effects of ACTH in the human being

The present paper is a report on a further metabolic study in a woman of 43 with rheumatoid arthritis

Nitrogen phosphorus calcium and sulfur analyses were made on diet urine and feces The metabolic periods were of 3 days duration Two control periods were followed by two periods during which ACTH 100 mg per day was administered This was continued through an additional 8 days during which 5 mg per kg body weight of aureomycin by mouth were given simultaneously with ACTH

Fig 7 shows the urinary excretion of nitrogen sulfur and phosphorus The changes in the fecal content of these elements were of little significance Nitrogen excretion increased markedly reaching a maximum by the third ACTH period (9 days) after which on aureomycin it fell Sulfur excretion showed similar behavior In contrast the phosphorus excretion increased with the first ACTH period and then decreased during subsequent periods to rise again in the last ACTH plus aureomycin period

Fig 8 shows the urinary nitrogen/sulfur and nitrogen/phosphorus ratios for the same periods In this chart the ratios are not corrected for urinary calcium Such corrections would not alter the picture

It is to be noted that the nitrogen/phosphorus ratio increased first in the second ACTH period and returned to pre treatment levels in the following ACTH and aureomycin period During this experiment there was progressive clinical improvement in this patient and the urine 17 ketosteroid excretion in mg per 24 hours was 5 4 in the control period 19–27 in the first two ACTH periods and 35–39 in the ACTH plus aureomycin periods In a later period (after all therapy was discontinued) the 17 ketosteroid level returned to 11 4 mg per 24 hours

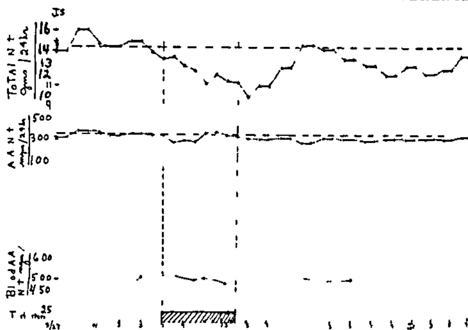


Fig 6

and elevation of fasting blood amino acid nitrogen levels The pattern of the amino acid nitrogen loss does not strictly parallel the pattern of total nitrogen loss

2 The ACTH induced catabolic reaction may be obtained without increased participation of the thyroid

3 Administration of thyrotrophin resulted in a catabolic reaction with enhancement of amino acid nitrogen excretion and elevation of fasting blood amino acid nitrogen level

4 A spontaneous anabolic phase of nitrogen metabolism may occur without participation of the testis

5 During testosterone induced anabolic states there is no significant alteration in fasting blood or urinary amino acid nitrogen

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2 Albanese A A and Irby V Determination of Urinary Amino Nitrogen by the Copper Method J Biol Chem 1944 153 583

excretion curves have occurred with remissions of the disease occurring with jaundice and with pregnancy We have been unable to reproduce this curve by high protein diets testosterone artisone adrenaline antihistaminics and various other steroids So far the excretion curve has occurred only with remission of the disease It was therefore decided to try histidine tolerance tests using injectible histidine with and without ACTH administration It was believed that

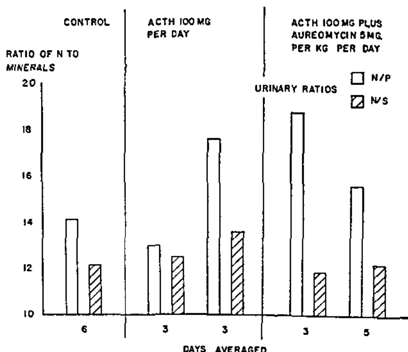


FIG 8 Urinary nitrogen/phosphorus and nitrogen/sulfur ratio

by injecting sufficient histidine a typical curve might be obtained and it was also hoped that such a tolerance test might reveal information as to the effect of ACTH on renal clearance

Histidine monohydrochloride equivalent to 500 mg histidine were given intramuscularly to one normal woman and one woman with rheumatoid arthritis Blood samples were obtained fasting and at ½ 1 2 and 4 hours following the injection Partitioned urine specimens were collected for 24 hours On the 3rd day following the tolerance test the administration of ACTH (10 mgs every 6 hours) was begun and on the 4th day of treatment a second histidine tol

The pattern of metabolic changes seen in this patient is the same as the findings made in three previous cases so studied

It follows that ACTH causes an increased urinary nitrogen and sulfur *excretion accompanied by a relatively reduced urinary ex cretion* of phosphorus This urinary phosphorus deficit is not ac counted for by fecal phosphorus and may perhaps be explained by glycogen deposition as has been postulated by Bartter et al

It also follows that aureomycin seems to counteract these meta

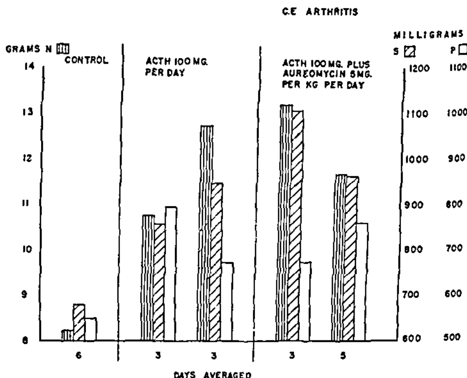


FIG 7 Excretion of nitrogen sulfur and phosphorus in urine

bolic effects without impairment of adrenal function as evidenced by continued clinical response to ACTH while aureomycin is being given and by increasing 17 ketosteroid levels in the urine during such therapy

DR W PAUL HOLBROOK (Tucson) The work I wish to discuss was car ried out by my associates A L Borden E C Brodie E B Wallraff and A R Kemmerer At the first ACTH Conference we reported the marked rise in urinary histidine excretion associated with remis sions of rheumatoid arthritis on the administration of ACTH or cor tisone Sixty patients on metabolic control were studied Similar

with the high urinary excretion of histidine occurring with remis sion plasma levels remained near the normal level as shown by the lower dotted line

It is of considerable interest that with 500 milligrams injected histidine no appreciable increase in urinary excretion was detectable

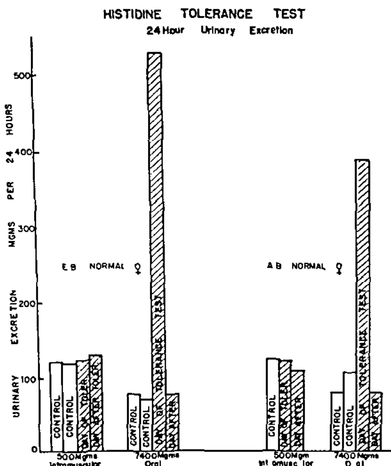


FIG 10

Furthermore even with the large oral doses only 4% and 6% respec tively could be accounted for in urinary excretion

We still feel that the increased histidine excretion which also in volves some of the other amino acids may have some relationship to the mechanism of remission We have no explanation as to what be comes of the histidine not accounted for in the urine These tolerance studies would appear to show that the curve is not due to a simple

erance test was run A normal diet containing 1 gm protein per kg body weight was adhered to throughout the test Microbiological assays were performed on all specimens at the conclusion of the experiment

The results of urinary histidine excretion are shown in Figure 9 While there were some small variations in the partitioned samples it is very clear that no major change occurred in the 24 hour excretion In spite of the fact that only minimal improvement was experienced by the patient with rheumatoid arthritis there was nev

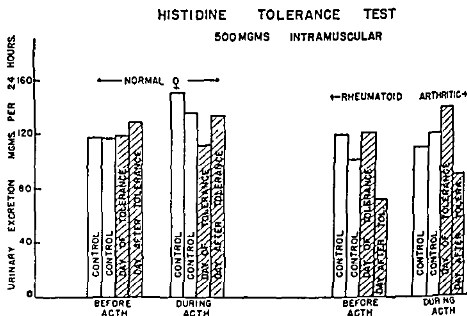


Fig 9

ertheless a change in the urinary excretion of histidine as compared with the normal

Because 500 milligrams of histidine failed to produce major urinary excretion it was decided to give a massive dose orally Accordingly 7400 milligrams of histidine was given in one dose to two normal individuals The results are shown in Figure 10 For the first time we were able to produce an increased urinary excretion comparable with the curve seen during remission This appeared to dispose of our theory that the histidine urinary curve might be related to remission However when plasma levels were estimated (Figure 11) it is clearly seen that there is an entirely different mechanism involved than with remission With the large oral dose plasma levels were tremendously increased resulting in high urine levels whereas

glycine for each 15 lbs of body weight Plasma glycine was determined at 20 80 140 and 200 minutes after the injection Four of the patients were then treated for 21 days with ACTH and three with cortisone Each of the 7 patients had glycine tolerance tests repeated after 10 and 21 days of treatment Glycine determinations were done by the method of Alexander Landwehr and Seligman as modified by Christensen and Shwachman

The normal range of fasting plasma glycine is from 1 47 to 2 83 mg %

Only two of the patients showed abnormally low values One was a man with very severe rheumatoid arthritis and severe malnutrition During treatment his appetite and food intake increased enormously and there was a gradual rise in his fasting glycine level The other had a low fasting level in the third test He showed good improvement for 10 days then developed abdominal pain nausea vomiting and anorexia during the second half of his period treatment and lost 14 lbs

The tolerance curves following the abnormally low fasting levels were slightly flattened but not nearly to the degree observed by Alexander

From our results it appears that there is a relation of the fasting glycine levels to the state of nutrition and food intake of the patient It should be noted that the fasting levels of the other five patients tended to decline during the treatment period

DR D M BERGENSTAL I wonder if we will be able to say anything regarding specific mechanisms for these observed effects until we have careful renal clearance techniques to determine whether the renal threshold is lowered

lowered kidney threshold for histidine Further tolerance studies on patients with rheumatoid arthritis are in progress

DR J SYDNEY STILLMAN (Boston Massachusetts) *I should like to add* the data we accumulated in the study of one amino acid glycine to that presented by Dr Bergenstal which concerned amino acid metabolism as a whole

The concentration of glycine in the blood plasma and erythro

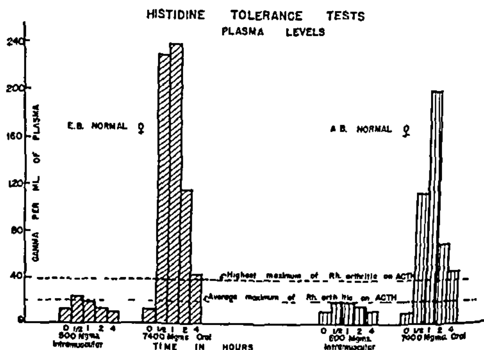


FIG 11

cytes was determined in a variety of pathological conditions by de Vries and Alexander in 1948 They also observed the effect of intra venously administered glycine in these patients Two of the six pa tients with rheumatoid arthritis studied showed abnormally low fasting levels and one of these had remarkably abnormal glycine tol erance curves Not only were they flat but they returned practically to the pre injection levels We attempted to confirm their results and also to observe the effect of ACTH and cortisone on the fasting glycine *levels and tolerance curves*

Fasting plasma glycine levels were done on 2 normal subjects and on 7 patients suffering from rheumatoid arthritis Then a sterile 10% aqueous glycine solution was administered intravenously 1 gram of

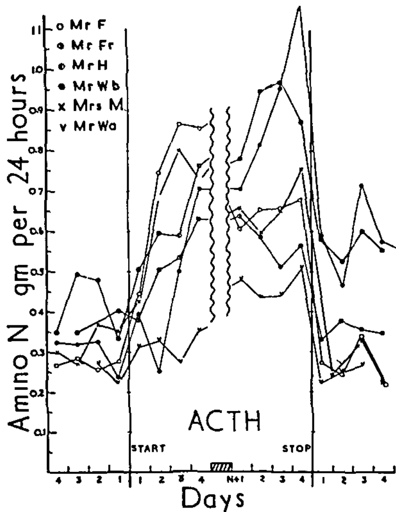


FIG 1 Amino nitrogen in urine (gms per 24 hours) during ACTH therapy

of ACTH for periods of eight to twenty four days The graph records the base line period the first four days of treatment the last four days of treatment and four days following treatment It will be ob served that the excretion of amino nitrogen rises sharply in the first 24 to 48 hours of therapy and maintains a high level until the treat ment is discontinued when it returns sharply to the original level The lowest line (Mr Wa) is the record of a patient receiving only 40 mgms of ACTH daily The others were given at least 100 mgms daily

The amino nitrogen/creatinine ratio has been followed in a num

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Studies on the Behaviour of Urinary Amino Nitrogen, Serum Alkaline Phosphatase and Pseudo Cholinesterase Activity During ACTH Therapy*

C J Bardawill,† A G Gornall M Nishikawara and K J R Wightman
UNIVERSITY OF TORONTO TORONTO

1 Urinary Amino Acids

The urinary amino acids in patients suffering from various diseases while under therapy with ACTH and cortisone have been studied as a part of a program to investigate total nitrogen metabolism as completely as possible The method used is that of Albanese and Irby[1] which has given quantitative recoveries (±5%) of the amino acids commonly present in urine in our hands The results in normal subjects are comparable with those obtained by the Van Slyke manometric method These patients have been maintained on a constant diet (P 80 F 90 CHO 275) with a salt content of either 2 or 5 grams and a constant purine level

The results have been expressed either as grams of amino nitrogen per 24 hours corrected by an appropriate amount if the creatinine excreted on that day varied significantly from the average or as an amino nitrogen/creatinine ratio Creatinine excretion remained reasonably constant in all patients but one who excreted smaller amounts whenever he was given a large dose of ACTH Results are reported in ten patients suffering from a variety of diseases and also an acute experiment in which six normal subjects were given 25 mgms of ACTH The amino nitrogen/creatinine ratios in urine secreted in four hours before and after the injection are compared Eosinophile counts are recorded

In figure 1 are *illustrated the values obtained* (grams of amino nitrogen per 24 hours) in six patients who received a constant dosage

* This investigation assisted by the Ontario Cancer Treatment and Research Foundation
† National Cancer Society Research Fellow

an effect on the equilibrium between this pool and the tissue proteins Further work bearing on this point is in progress

2 Serum Alkaline Phosphatase

Serum alkaline phosphatase activity has been studied in sixteen patients eleven of these received ACTH and five receiving cortisone therapy Ten of the above patients suffered from leukaemia or lymphoma

The method was a modification of the Bodansky and Kay Jenner procedure using a sodium β glycerophosphate substrate at pH 9 2 and a three hour incubation period In addition studies were carried out in ten patients using the above substrate containing 001 molar sodium cyanide The results are expressed as Kay Jenner units

The changes which have been observed in serum phosphatase ac

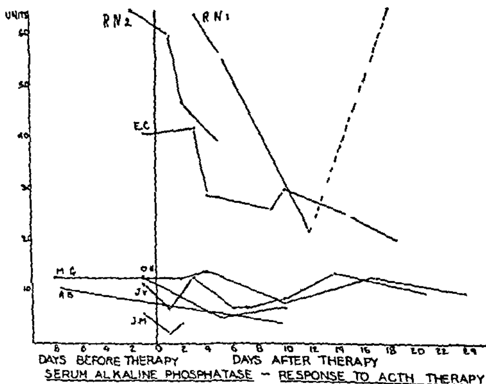


Fig 2 Serum alkaline phosphatase activity in patients showing clinical improvement on ACTH therapy (Kay Jenner units) Note that there is no alteration in cases where the alkaline phosphatase activity is in the normal range in the pretreatment period

ber of patients treated for long periods with varying doses of ACTH or cortisone It was noted that sharp initial increases in the ratio occurred but wide fluctuations occurred as therapy was continued and as the dosage was altered In two instances the prolongation of effect by a depot of cortisone was observed

Table 1 shows the response of six normal individuals to a single injection of 25 mgms of ACTH The amino nitrogen/creatinine ratio increases in all cases a change which seems more significant when it is noted that most subjects excreted a lower ratio after an injection

Table I

EFFECT OF 25 MGM ACTH ON EOSINOPHILES AND AMINO NITROGEN/CREATININE RATIO

	Eosinophile Count		Amino Nitrogen/Creatinine Ratio (Urine)	
	Before	4 Hours After	Before	4 Hours After
Normal Subjects				
H G	610	170	18	28
B M	126	55	26	30
N H	137	16	25	32
M T	115	24	17	21
J C	559	126	12	15
A M	66	33	17	20
Control Series—1 cc Saline				
H G	407	363	13	17
B M	93	115	28	18
N H	165	82	30	20
M T	77	49	21	15
J C	181	154	16	13
A M	66	33	19	17

of saline instead of ACTH The experiment was carried out on two successive days giving one half of the group ACTH and the other saline and reversing the procedure the next day Further study on the behaviour of normal subjects seems to be indicated

Discussion

The excretion of amino nitrogen which has been illustrated here is only a small part of the total nitrogen excretion—usually less than 10% In general it appears to parallel the total nitrogen excretion Since we have not yet undertaken the study of amino nitrogen in plasma interpretation of the results is not possible but it is felt that they probably indicate a flooding of the metabolic nitrogen pool by

acute leukaemics who had a full haematological remission as a result of therapy showed a slower rate of disappearance of radioactive phosphorus as treatment progressed

The third patient who had hemolytic anaemia did not show either physiological or therapeutic response to ACTH The occurrence of abnormal alkaline phosphatase enzymes which are resistant to physiological growth inhibiting hormones is suggested by the above data The effect of cortisone on serum phosphatase activity is shown in figure 4

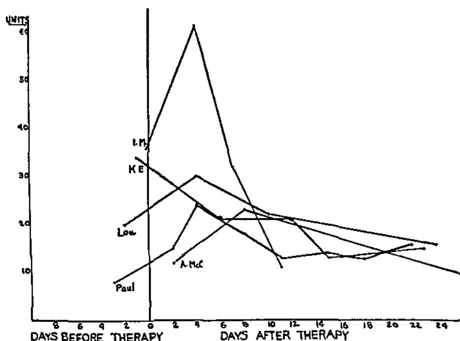


FIG 4 Serum alkaline phosphatase changes during cortisone therapy

The behaviour of the fraction of phosphatase which is not inhibited by cyanide was also followed and not found to be influenced by therapy

Discussion

Phosphatase is particularly plentiful in cells engaged in protein synthesis[28] In embryonic tissues the phosphatase reaction is weak during the early stages of embryonic development but becomes intense at the beginning of differentiation when synthesis of new pro

tivity have been somewhat variable but in the majority of instances there has been a tendency to suppression of the enzyme activity following ACTH or cortisone administration although an initial rise in serum phosphatase activity was noted in some instances when the *latter hormone was given*

As seen in figure 2 the patients with a normal enzyme activity did not change appreciably Two patients one with chronic myeloid leukaemia and one with Hodgkin s disease had high levels which fell

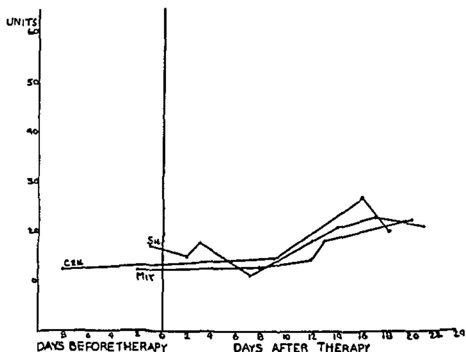


FIG 3 Serum alkaline phosphatase in patients who did not improve on ACTH therapy

markedly as a result of treatment One of these rose again when treatment was stopped and fell once more on a second course of therapy

Contrasted with these are the patients shown in figure 3 who showed a rise Two of these are acute leukaemics who showed no clinical response to therapy whatever This is of interest since they are the only two patients who failed to improve at least subjectively This change was found to be associated in one patient with a marked increase in the rate of disappearance of a tracer dose of radioactive phosphorus from *the plasma late in therapy as compared to a similar* test at the beginning The same procedure carried out on one of the

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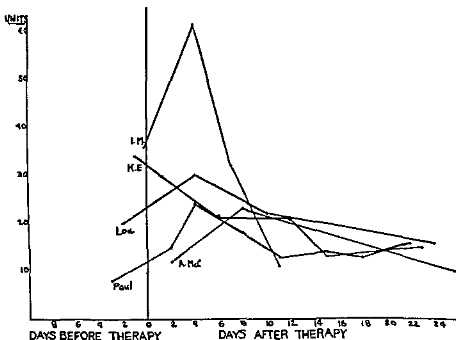


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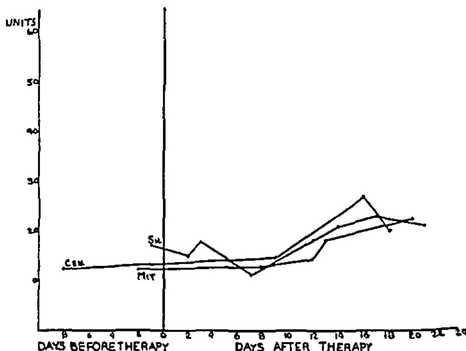


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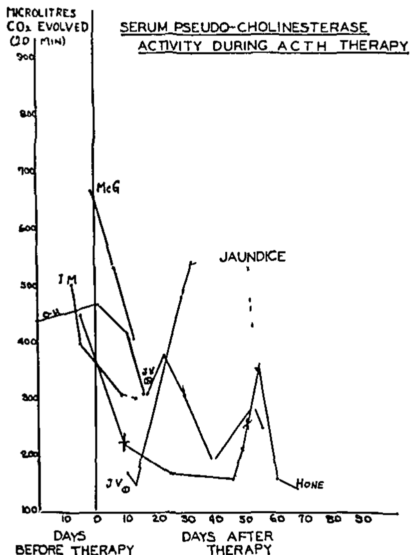


FIG 5 Plasma pseudo cholinesterase activity during ACTH therapy

nitrogen balance Studies of the effect of simultaneously administered androgen are in progress

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tein at the expense of the yolk is active[3] The finding of Li Kalman Evans and Simpson that ACTH decreases the phosphatase activity of rat plasma[4] and that this decrease can be checked by the simultaneous administration of growth hormone[5] suggest that ACTH may interfere with these reactions

The anomalous behaviour of the serum phosphatase activity in *the patients not improved by therapy seemed worthy of note*

3 Serum Pesudo Cholinesterase Activity

The enzyme activity was measured manometrically by Annon s adaptation of Warburg s procedure at pH 7 4 and 37 5 C using 0 025 M sodium bicarbonate saturated with 5% carbon dioxide in nitrogen The final concentrations of the substrate for true and pseudo-cholinesterase activity were 0 03 M mecholyl and 0 006 M *benzoyl choline* respectively *The results were expressed in terms of* microliters of CO_2 evolved by one millilitre of plasma after 20 minutes incubation at 37 5 C The enzyme activity was determined before therapy was begun and followed at approximately weekly intervals during therapy

The results are shown in figure 5 A fall in activity occurs in all cases Two of the patients became jaundiced during therapy with a fall occurring at or before the period of maximum jaundice and a rise as the jaundice recovered The patient J V is one of these and in a *second course of therapy also recorded in the graph* he follows the more usual course

Discussion

The range of pseudo-cholinesterase activity in normals is said to range from 360 to 920 but an individual tends to hold a constant level The nature of the illness these patients had has prevented us from doing prolonged base line studies and little is known about the fluctuations of plasma cholinesterase activity in hematological disorders Attempts have been made to correlate changes in plasma cholinesterase activity with certain pathological conditions but outside of thyotoxicosis hepatic damage and diseases associated with generalized wasting and inanition there is no definite evidence for any such relationship[6]

Sawyer and Everett[7] have presented evidence that the level of pseudo cholinesterase in the plasma of rats parallels the estrogen level of these animals and it is possible that findings of falling pseudo-cholinesterase values during ACTH therapy reflect alterations in the estrogen androgen ratio of the blood and/or the degree of negative

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An Inquiry into the Specificity of the Uric Acid Creatinine Ratio as a Measure of Adrenal Cortical Responsiveness*

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This report is concerned with the specificity of the uric acid creatinine ratio as a measure of adrenal responsiveness

The optimal use of hormonal preparations such as ACTH is dependent in large measure on the availability of specific indices by which their effects can be quantitatively evaluated Among the criteria proposed for assessing ACTH activity is that introduced by Forsham Thorn and their associates[1] which utilizes changes in the urinary uric acid-creatinine ratio as a measure of adrenal responsiveness These workers report for normal subjects an increase in uric acid creatinine ratios following 25 mg ACTH which range from 62 to 130% average +91% For Addisonians the average rise was +16% with a range from −14 to +58% The increased uric acid excretion following ACTH was regarded as due either to increased production increased clearance or both It was suggested that the reliability of this test might be reduced in the presence of certain pathological conditions such as gout leukemia hepatic cirrhosis renal insufficiency or hypothyroidism conditions which either effect renal excretion of uric acid or its endogenous metabolism

Our interest in this test as a measure of adrenal responsiveness was stimulated by unpredictable and significant variations encountered in normal subjects following ACTH administration In an analysis of the factors which might be responsible for this variability control observations were made in the fasting state alone conditions under which this test is carried out omitting however, the administration of ACTH

* Aided by a grant from the Playtex Park Research Institute The ACTH used in this study was generously supplied by Dr John R Mote of the Armour Laboratories We wish to acknowledge the technical assistance of Connie Sonn Lucille Davis and Olga Rochovansky

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day 25 mg of ACTH were administered with a 90% increase in the ratio and a 57% drop in eosinophils It was evident that the fasting procedure utilized in this test produced per se an elevation in the uric acid-creatinine ratio in this normal subject of the same order of magnitude as that following ACTH administration

The next two fasting experiments which were carried out 10 days later showed a much higher basal uric acid-creatinine ratio and hardly any rise in the 9 a m to 12 noon specimen Inspection of the data suggested the possibility that the variations in the ratios might be related to the level of uric acid excretion during the control period We therefore modified the basal level of the uric acid excretion by varying the purine intake of this subject

In the last experiment the control subject took 2 high purine meals consisting of about 200 grams of sweetbread for lunch and sardines for supper on the day preceding the test This resulted in a high basal excretion of uric acid and a total absence of any rise in the ratio following ACTH despite the fact eosinophils fell to the same extent as in the previous ACTH test

Figure 2 shows similar observations in other normal controls The

Uric Acid/Creatinine Ratio in Normal Controls

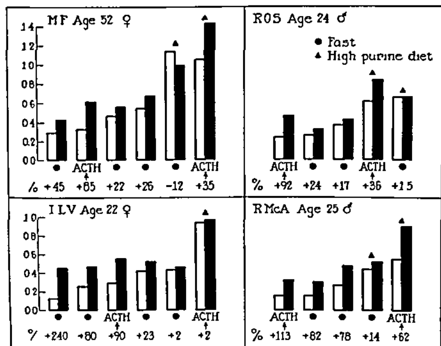


FIG 2

In all about 70 tests were carried out in the fasting state alone and following ACTH in 7 normals 4 Addisonians and 5 patients with miscellaneous diseases Representative data are presented which bear on the problem of the variability of the uric acid creatinine ratios observed

In Figure 1 are shown the uric acid creatinine ratios and the eosinophil counts in one normal control under various conditions The open columns represent the ratio in the 6 to 8 a m specimen the

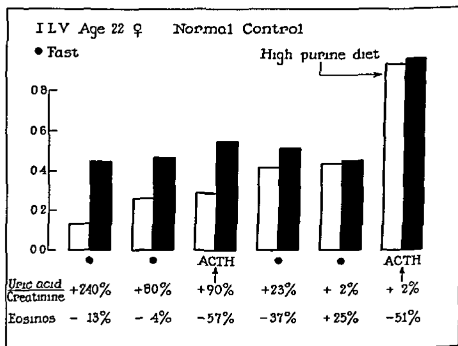


FIG 1 Uric acid creatinine ratios in one normal control

solid blocks the ratio in the 9 a m to 12 noon specimen The first row of figures shows the percent increase in the ratio below which is given the percent drop in eosinophils The circles represent tests carried out with fasting alone In the first experiment no ACTH was given the control specimen was collected from 6 to 8 a m and blood then drawn for eosinophil count Another specimen was collected from 9 a m to 12 noon followed by a second eosinophil count There was a 240% increase in the uric acid-creatinine ratio but only a small drop in eosinophils The next experiment was carried out on another day under the same conditions there was an 80% increase in the ratio and again only a small change in eosinophils The following

ease In 3 of these patients ACTH as well as fasting alone failed to elevate the uric acid-creatinine ratio significantly However in one patient in the lower right corner fasting resulted in a 105% rise in the ratio and the administration of ACTH in a 52% rise There was no appreciable drop in eosinophils in any case

Another observation was that in a very high percentage of normal controls as well as Addisonians the administration of ACTH as well as fasting alone was followed by an increase in urinary pH (Fig 4)

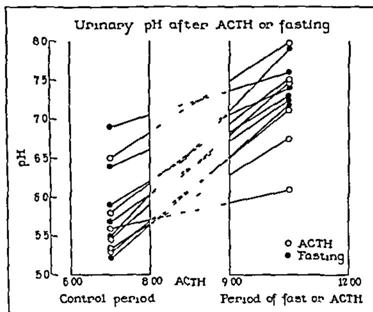


Fig 4

It has been shown that the urinary excretion of citric acid is a function of the urinary pH increasing as the pH rises and diminishing with a fall in urinary pH [2,3]

These findings prompted a study of the citric acid creatinine ratio following fasting with or without ACTH with results that are shown in Figures 5 and 6

Figure 5 gives data on the same 4 normal controls It is apparent that in addition to the effect of fasting and ACTH on the uric acid creatinine ratios these conditions also cause a rise in the citric acid creatinine ratio Furthermore just as with the uric acid creatinine ratio the basal level of citric acid excretion determines the extent of the rise both to fasting and to ACTH Thus in patient M F an ACTH response of +87% was reduced to +33% and a fasting re

relationship is evident between the level of the basal excretion of uric acid and the subsequent rise in the ratio following either fasting or ACTH Thus with patient M F an ACTH response of 85% in the ratio was reduced to +35% when the basal uric acid excretion was elevated by a high purine diet as in the tests marked with the solid triangle A +45% increase in the uric acid ratio on fasting alone with a low basal uric acid output was converted to a depression of 12% when the basal uric output was elevated Similar findings were obtained in the other cases shown in this slide both with respect to the response to fasting alone as well as to ACTH

It is apparent that the basal uric acid creatinine ratio which is influenced chiefly if not entirely by the uric acid excretion inasmuch as urinary creatinine is quite constant from period to period is an important determinant of the response not only to fasting but to ACTH as well Secondly the response to fasting *per se* may constitute a considerable portion of the response made to the administration of ACTH and thirdly the influence of the basal uric acid excretion may be sufficient to reduce the ACTH response in normal subjects to the range regarded as indicative of adrenal unresponsiveness

In Figure 3 are shown the data in 4 patients with Addison s dis

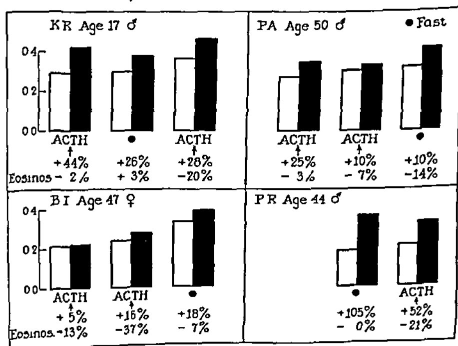


FIG 3

shows the influence of the basal excretion level on the subsequent response Thus with ascorbic acid the rise in the ascorbic acid-creatinine ratio seen during the first test on fasting alone failed to occur on the second test when the basal ascorbic acid excretion level had risen from 0 023 to 0 040 On the other hand a greater rise in the creatine-creatinine ratio was seen in the second test carried out when the basal ratio had fallen from 0 06 to 0 04

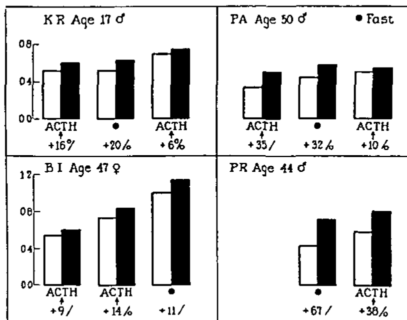


Fig 6

SUMMARY

These results which relate only to the test procedure and time intervals as described by Forsham Thorn and their associates may be summarized as follows

1 Fasting *per se* causes a rise of considerable magnitude in the uric acid-creatinine ratio in normal subjects The extent of the rise is generally not as great as after the administration of ACTH but a variable yet appreciable portion of the rise in the ratio following ACTH can be attributed to the fasting conditions *per se* under which the test is carried out

2 In addition to the uric acid other urinary constituents are ele

sponse of +87% was reduced to +9% by higher basal citric acid excretion It should also be pointed out that deviations in the citric acid creatinine ratio will occur not only on the basis of the basal citric acid excretion but in proportion to the rise in the urinary pH in the course of the test This could be readily demonstrated in experiments not shown here by the administration of sodium bicarbonate at 8 a m a procedure which greatly enhanced the rise in citric acid creatinine ratio

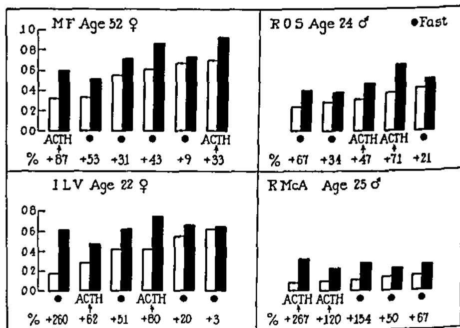


FIG 5

Figure 6 shows the urinary citric acid-creatinine ratio in the 4 Addisonians studied As with the uric acid creatinine ratios the increases in the citric acid creatinine ratios were of minor magnitude with the exception of subject P R who also showed a greater increase in uric acid-creatinine ratios than is considered typical of Addison s disease

Figure 7 includes data in one normal control not only with respect to uric acid and citric acid but also ascorbic acid and creatine which we have also found will change in their ratios to creatinine following fasting and ACTH A very considerable portion of the rise in the ratios of these latter urinary constituents after ACTH can also be attributed to fasting *per se* Inspection of the fasting data likewise

6 These observations are pertinent to the interpretation of the uric acid-creatinine ratio as a measure of adrenal responsiveness

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DISCUSSION

DR GREGORY PINCUS I would like to comment on the reverse experiment which has been done with rats in our laboratory by Dr Romanoff He studied allantoin excretion in adrenalectomized rats and what he found was a reduced excretion of allantoin in adrenalectomized animals but when an allantoin tolerance test was undertaken the rate of excretion of allantoin was not significantly different from that of normal animals

In other words there was no renal threshold effect and therefore I wonder if what Dr Taussky is talking about in the case of the fasted individuals is essentially a secretion of adrenal steroid which caused the uric acid effect observed

DR JACK METCOFF I would like to congratulate Dr Taussky Dr Swan and Dr Shorr on what seems to me to be a most interesting paper in terms of mechanisms

I have only two questions no cases to present The questions are these

First was Dr Taussky measuring total chromogens in terms of creatinine as the Taussky method does and second did she happen to do simultaneous endogenous creatinine clearances?

DR HERTHA H TAUSSKY I only want to re emphasize the fact that if a normal subject raises his basal uric acid excretion by two high purine meals the preceding day it is easily possible for the test to give results which would conventionally be interpreted as indicating adrenal unresponsiveness This potential error should be borne in mind when using the uric acid creatinine ratio as a diagnostic index

vated by ACTH and by fasting alone These include citric acid as corbic acid and creatine There is uniformly as well a considerable rise in urinary pH

3 In Addison s disease the response on the part of these urinary constituents to fasting and to ACTH is reduced Whenever ACTH failed to elevate the uric acid creatinine ratio significantly fasting also failed to do so

4 The extent of the increase in the uric acid creatinine ratio fol

D H (m) age 15 NORMAL CONTROL

Test	Collection Period	pH	RATIO TO URINARY CREATININE				EOS/mm^3
			Uric Acid	Citric Acid	Ascorbic Acid	Creatine	
	A M						
ACTH	6-8	6 3	0 35	0 09	0 07	0 10	365
	9-12	7 2	0 69	0 43	0 16	0 16	93
			+ 97%	+ 370%	+ 128%	+ 60%	- 75%
FAST	6-8	5 7	0 34	0 12	0 023	0 06	208
	9-12	7 5	0 55	0 30	0 034	0 13	175
			+ 62%	+ 150%	+ 48%	+ 117%	- 15%
FAST	6-8	5 7	0 33	0 11	0 040	0 04	
	9-12	6 7	0 51	0 29	0 040	0 11	
			+ 55%	+ 160%	0%	+ 175%	

Fig 7

lowing fasting or ACTH appears to be dependent upon the level of uric acid excretion during the control period When this is low the elevation of the ratio with both fasting alone and ACTH is accentuated When this is high the ratio is elevated to a lesser degree or not at all This phenomenon may provide an explanation for the variability in the response of the uric acid creatinine ratio from time to time in the same patient following ACTH and fasting

5 The concurrent changes in citric acid ascorbic acid and creatine excretion which accompany the rise in uric acid after ACTH suggest a renal mechanism for the effect not only of ACTH but also of fasting in the direction of an inhibition of tubular resorption of uric acid and the other urinary constituents studied

after injection of 25 mg of ACTH Armour standard) correspond to the conditions of Thorn s test of the eosinopenic response to ACTH [1]

Two cc of citrated plasma were dialysed for 48 hours against 500 cc of a veronal citrate buffer of pH 8 6 ionic strength $\mu = 0\ 1$ The dialysed plasma was then diluted with 0 55 cc of distilled water and made up to 7 0 cc with the above mentioned buffer The electro phoresis was done in an electric field of 8 80 Volt/cm at 0° C for 80 minutes The addition of distilled water to the diluted and dialysed plasma reduced the usual salt anomalies to such an extent that the ascending boundaries which are known to give a better resolution can be used for the analysis of the protein patterns Indeed under the conditions described above (similar to those of Longsworth[2] and Wiedemann[3]) the relative amounts of the proteins as well as their mobilities are identical in the ascending and descending boundaries The Longsworth screening method with two scanning knife edges (Perkin Elmer apparatus) was used and the patterns were evaluated according to the method of Tiselius and Kabat [4]

RESULTS

In the plasma of 7 out of the 45 patients more or less striking changes in the β globulin content occurred These observations which are illustrated in Figs 1–5 may be classified into two different phenomena These occurred in succession in one and the same pa tient

1 The β globulin decreased during ACTH therapy in 6 of these patients (C B G B H C D B R S and E R) This was the most striking in a case of cancer of the sigmoid (C B Fig 1) and also marked in a case of sarcoma of the kidney (D B) The latter observa tion is illustrated by the electrophoretic patterns shown in Fig 2 In *both instances these decreases occurred during the four hours follow* ing the injection of 25 mg of ACTH Armour

2 A marked increase in the β globulin content occurred *after ces sation of ACTH therapy* in 5 patients (R S E R J M H C and D B) with the following diagnoses rheumatoid arthritis (Still s dis ease) in a boy of 7 years (R S Fig 3) Marie Strumpell s disease in a young woman of 30 (E R Fig 4) multiple myeloma in a man of 48 (J M) and diabetes and cerebrovascular accident in a man of 57 (H C) In the fifth case a man of 50 with sarcoma of the kidney (D B) it was not established whether the increase occurred actually after cessation of ACTH therapy

While the fall in the β globulin content occurring during ACTH therapy was rapid i e within four hours after a single dose of 25 mg *the rise of β globulin developed more slowly in one case during ta*

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ACTH and Plasma Proteins*†

Peter Bernfeld Charles D Bonner and F Homburger

CANCER RESEARCH AND CANCER CONTROL UNIT AND JEWISH MEMORIAL HOSPITAL TUFTS COLLEGE MEDICAL SCHOOL, BOSTON

In the course of electrophoretic studies on the distribution of plasma proteins in patients receiving ACTH we have observed significant changes in the β globulin content These observations are of interest because it is generally believed that ACTH has the effect of altering especially the γ globulin

METHODS AND PATIENTS

Electrophoretic analyses of plasma were done at varying time intervals before during and after administration of ACTH (Armour Searle and Wilson)

Some 300 experiments were done on repeated samples taken from 45 patients These patients suffered from the following diseases cancer (17) including synovioma multiple myeloma and lymphoblastoma rheumatoid diseases (14) including one case of Marie Strumpell s disease and one of Still s disease asthma (1) diabetes (1) and skin diseases (3), namely pemphigus exfoliative and seborrheic dermatitis There were 5 cases of cerebrovascular accident one myasthenia gravis complicated by rheumatoid arthritis and acquired hemolytic jaundice one fractured hip and one case of infectious mononucleosis and unspecific carditis

These patients 24 women and 21 men ranged in age from 7 to 94 years The dosage of ACTH ranged for a single dose of 25 mg to a total amount of 20 Gm of ACTH given during one year

Whenever possible the blood specimens were taken in the fasting state The specimens for the short tests (taken before and four hours

* This study was aided by the grants from the American Cancer Society Inc. New York and the Damon Runyon Memorial Fund for Cancer Research

† The ACTH used in this work was provided by the Armour Laboratories and in part purchased with funds received from the United States Public Health Service National Cancer Institute Bethesda Maryland Some ACTH was obtained through the Searle Laboratories and the Wilson Laboratories Chicago Illinois

In 4 of these 7 patients both phenomena (β globulin increase and β-globulin decrease) occurred successively In all these instances the decrease took place during therapy the increase following therapy

In one single case (H C) the α-globulin content increased during ACTH therapy followed by a return to normal after therapy

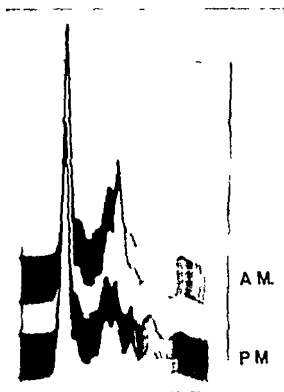


FIG 2 D B Neurogenic sarcoma of kidney man aged 50 Ascending boundaries before and four hours after test dose of 25 mg ACTH Armour From left to right the peaks represent albumin α_1 α_2 α_3 β globulin fibrinogen and γ-globulin

whereas the distribution of the other proteins did not vary in these 7 patients

In the remaining 38 patients no major changes in the plasma protein content were encountered with the exception of one single case (pemphigus) [5] where the γ-globulin decreased during ACTH therapy and then increased to pre therapy level when ACTH administration was interrupted This effect however was less marked than the changes of β-globulin reported A lack of a change in the γ globulin content is particularly conspicuous in the case of multiple myeloma

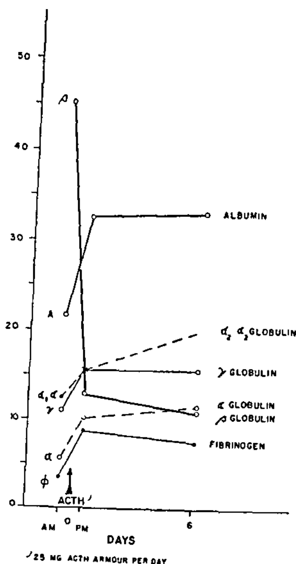


Fig 1 C B Cancer of sigmoid man aged 60 Proteins expressed in relative amounts One test dose of 25 mg ACTH Armour

pering off the dosage of ACTH (J M) or in general after cessation of therapy In one case the peak of β globulin was reached on the 75th day after therapy was discontinued (R S) In two cases where the study was continued long enough the abnormally high β globulin levels returned to normal without clinical changes or therapy (R S and E K) The successive electrophoretic patterns obtained in R S are shown in Fig 5

esis is particularly attractive because it has been shown that serum β-globulin in the rabbit may increase with cholesterol feeding [6]

While the effects of ACTH on the distribution of plasma proteins are usually quoted as concerning the γ globulins such a change was observed in only one single case in 45 patients Even in the patients with as much as 20% of γ globulin this high content was not depressed by ACTH therapy *We can not explain the absence of changes*

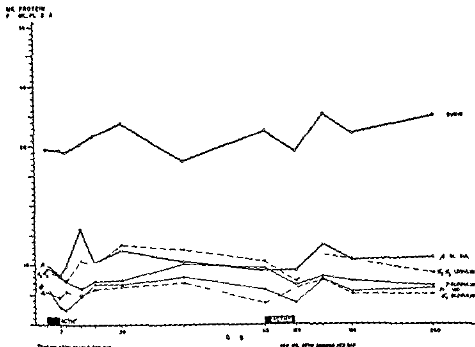


FIG 4 E R Marie Strumpell s disease woman aged 30 Proteins expressed in mg per ml plasma First course 200 mg ACTH Searle Second course 100 mg ACTH Armour per day for 16 days Lot No H 7811

in the γ globulin content of the plasma of most of our patients nor do we know why the changes in β globulin content occurred in only 15% of the cases

SUMMARY

1 Marked *decreases* of plasma β-globulin occurred in patients during administration of ACTH

2 Marked *increases* of plasma β globulin occurred *after* cessation of ACTH therapy

(J M) where noticeable increase of β globulin is not accompanied by any definite change in the γ globulin boundary as well as in other patients with high γ globulin content

GENERAL DISCUSSION

The data show that there are actual increases and decreases in the amount of circulating β globulin associated with the administration

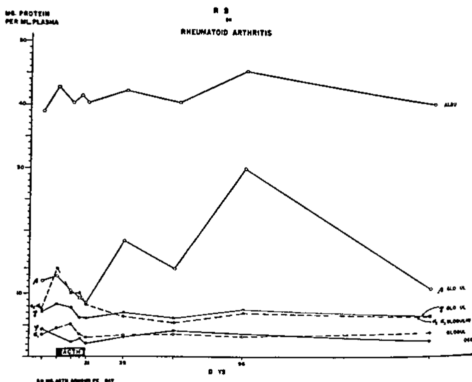


Fig 3 R S Still s disease boy of 7 years Proteins expressed in mg per ml plasma Received 60 mg ACTH Armour per day for 14 days Lot No H 7511

and discontinuance of ACTH There was no evidence indicating any relationship of these changes to a disease state Furthermore they do not parallel any clinical phenomena occurring in these patients There is also no apparent relationship of such changes to the brand lot or dosage of ACTH given

It is conceivable that the decrease of β-globulin under ACTH therapy and its slow increase thereafter may be in some way con nected with lipid metabolism since it is known that a high propor tion of lipoproteins is present in the β globulin group This hypoth

6 Fishberg A M L Friedfeld J Hoffman E R Stoller and E H Fishberg Proc Soc Exper Biol & Med 75 301 1950

DISCUSSION

DR S HOWARD ARMSTRONG JR The diagrams in the paper under discussion are technically excellent and I have no question about the actual changes shown I do have some question in mind as to their significance in terms of the lipid and protein components which move in electrophoretic diagrams with the mobilities of alpha and beta globulins

The interpretation of electrophoretic diagrams with respect to these globulins is a very difficult matter Thus we know that in certain sera of the nephrotic syndrome which can be shown to have large quantities of materials which have particle size characteristic of β lipoproteins by osmotic pressure and ultracentrifugal measurements there is a great increase in the α peak With improvement of the nephrotic syndrome as the amount of β lipoproteins appears to decrease by methods other than electrophoresis a relative increase can occasionally be shown by electrophoresis

On the basis of experience of this kind in putting together electrophoretic data with data from other methods in protein characterization I am puzzled as to the meaning of the changes shown

In this connection it may be of interest that with some of the very first ACTH which we had in Dr Thorn s clinic three years ago we did acute experiments on the effect of ACTH on the electrophoretic schlieren diagram without noting any significant changes and for this reason did not publish our work Of course the work of the paper just presented represents much longer term observations

DR PETER BERNFELD The variations of β globulin content were the same whether measured in the ascending or in the descending boundaries and were independent of the usual plasma β globulin anomaly of the descending boundary The changes in the β globulin content observed by electrophoresis are accompanied by corresponding alterations in the total plasma nitrogen (Kjeldahl) The changes observed must therefore be considered to be real ones We did not carry out any salt fractionations on these plasma samples

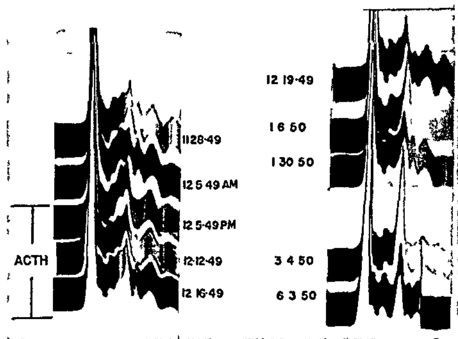


FIG 5 R S Refer to Fig 3 Electrophoretic patterns ascending boundaries on dates indicated Note ACTH medication from 12 5 49 to 12 16 49

3 Only 15% of the patients show these changes which sometimes occur successively in one and the same patient

4 No relationship is evident between the changes in the plasma protein distribution and the patient s disease

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the average daily renal excretion of purines in the five dogs during the control period The excretion of total purine nitrogen (sum of allantoin nitrogen and uric acid nitrogen) per kilogram of body weight was approximately the same for all dogs regardless of breed or weight The excretion of uric acid per kilogram was nine times greater in the Dalmatian than in the mongrel dogs On the other hand the allantoin excretion per kilogram was three times greater in the mongrels

345 mg of ACTH per day were administered to all dogs for pe

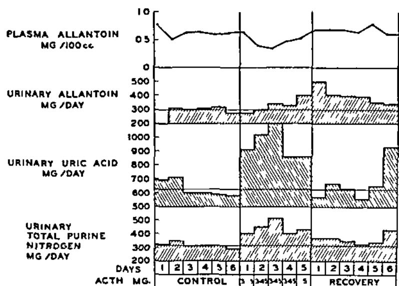


Fig 1 Effect of ACTH on plasma allantoin and on renal excretion of total purine nitrogen uric acid and allantoin

riods of 4–6 days Figure 1 shows the effect of ACTH on the renal excretion of purines in Dalmatian 1 There was a significant increase in excretion of uric acid during the period of administration of ACTH The greatest increase in excretion of allantoin occurred dur ing the recovery period There was an appreciable increase in total purine nitrogen excretion

Similar data were obtained for the second Dalmatian In this dog the quantitative response to ACTH in renal excretion of purines was less intense During the first day of ACTH there was a marked fall in the concentration of plasma uric acid The increased renal excretion of uric acid on that day may therefore be attributed to increased renal

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ACTH-Induced Changes in Purine and Carbohydrate Metabolism in Dalmatian and Mongrel Dogs

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UNIVERSITY OF MICHIGAN MEDICAL SCHOOL ANN ARBOR

A relationship between ACTH induced diabetes in man and the associated changes in purine metabolism has been postulated It has been reported by others that carbohydrate metabolism in mongrel dogs is not affected by large doses of ACTH Because of the recognized differences in purine metabolism between pure bred Dalmatian and mongrel dogs a comparative study was made of the effects of ACTH upon both purine and carbohydrate metabolism in the two strains

Two Dalmatian and three mongrel dogs were used Table I shows

Table I

RENAL EXCRETION OF ALLANTOIN AND URIC ACID AND OF TOTAL PURINE NITROGEN PER KILOGRAM OF BODY WEIGHT (Averages for Control Periods)

Dog	*Weight* kg	*Allantoin* mg/day/kg	*Uric Acid* mg/day/kg	*Total Purine Nitrogen** mg/day/kg
Dalmatian				
No 1	19 1	15 9	33 0	16 5
No 2	19 8	18 2	36 2	18 8
Average		17 4	34 6	17 7
Mongrel				
No 5	11 6	56 2	4 1	21 4
No 3	11 3	54 2	2 4	20 2
No 7	20 3	47 0	5 2	18 5
Average		52 5	3 9	20 0

* Sum of allantoin nitrogen and uric acid nitrogen

Research Fellow in Medicine of the American College of Physicians 1949-1950

the average daily renal excretion of purines in the five dogs during the control period The excretion of total purine nitrogen (sum of allantoin nitrogen and uric acid nitrogen) per kilogram of body weight was approximately the same for all dogs regardless of breed or weight The excretion of uric acid per kilogram was nine times greater in the Dalmatian than in the mongrel dogs On the other hand the allantoin excretion per kilogram was three times greater in the mongrels

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DALMATIAN 1 19 1 KGS

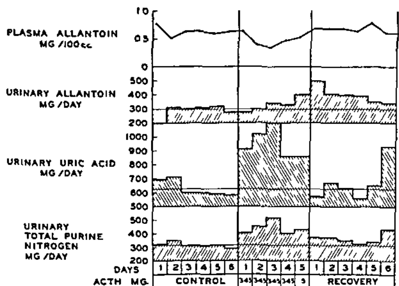


FIG 1 Effect of ACTH on plasma allantoin and on renal excretion of total purine nitrogen uric acid and allantoin

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Similar data were obtained for the second Dalmatian In this dog the quantitative response to ACTH in renal excretion of purines was less intense During the first day of ACTH there was a marked fall in the concentration of plasma uric acid The increased renal excretion of uric acid on that day may therefore be attributed to increased renal

clearance On the following days there was a rise in the concentration of plasma uric acid particularly during the last day of ACTH administration Since renal excretion of uric acid and total purine nitrogen remained elevated an increase in uric acid production must have taken place An increase in allantoin excretion did not occur until the recovery period

Figure 2 demonstrates the results obtained with mongrel dog 5 It can be seen that the rise in excretion of total purine nitrogen was

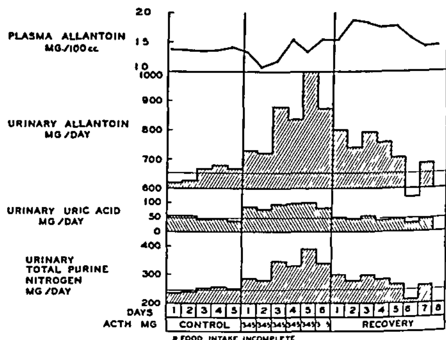


FIG 2 Effect of ACTH on plasma allantoin and on renal excretion of total purine nitrogen uric acid and allantoin

principally due to the rise in allantoin excretion With ACTH administration the plasma allantoin level first decreased then rose simultaneously with the increase in excretion of allantoin Both remained elevated during part of the recovery period It can be assumed that increased production of allantoin must have been present during both the ACTH and recovery periods The increase in uric acid excretion was not great and was present only during ACTH administration

In mongrel dog 3 the plasma concentration of allantoin did not exceed control levels until the recovery period It reached a maximum on the fourth recovery day the day of maximal renal excretion of allantoin This is evidence for increased production of allantoin

during the recovery period The elevated plasma concentration and urinary excretion of allantoin persisted through the fifteenth recovery day The concentration of uric acid in the plasma remained unchanged

The results obtained with mongrel dog 7 during and following ACTH administration were similar to those in mongrel 3 In mongrel 7 the increased production of allantoin during the recovery period persisted for at least nine days

There was a decrease in the uricolytic index of all dogs during ACTH administration This suggests that ACTH either depressed the uricase enzyme system or altered the manner in which the kidney regulates excretion of either or both of the purine end products

Table II

RISE IN RENAL EXCRETION OF ALLANTOIN AND URIC ACID AND OF TOTAL PURINE NITROGEN DURING ADMINISTRATION OF ACTH (345 MG/DAY) EFFECT OF ACTH ON GLUCOSE TOLERANCE

Dog	*ACTH mg/kg*	*Rise in Allantoin mg/day/kg*	*Rise in Uric Acid mg/day/kg*	*Rise in Total Purine Nitrogen* mg/day/kg*	*Effect on Glucose Tolerance*
Dalmatian					
No 1	18 1	1 6	16 5	6 3	++++
No 2	17 6	−1 8	11 7	3 2	+++
Mongrels					
No 5	30 0	16 0	3 5	7 0	0
No 3	30 5	9 0	7 0	5 6	+++
No 7	17 0	6 0	8 2	5 0	+

* Sum of allantoin nitrogen and uric acid nitrogen

Following ACTH administration glucose tolerance tests were performed in all dogs Table II shows a rough correlation between the increase in uric acid excretion produced by ACTH and changes in carbohydrate metabolism The greatest loss of glucose tolerance occurred in Dalmatian 1 (Figure 3) which also had the greatest increase in excretion of uric acid per kilogram of body weight Dalmatian 2 which had a rise in purine nitrogen little more than half that of the other dogs had the second highest increase in excretion of uric acid per kilogram A moderately severe loss of glucose tolerance was seen in this dog No change in carbohydrate tolerance was seen in mongrel 5 (Figure 4) which had the smallest rise in the excretion of uric acid per kilogram of all dogs although it had the greatest increase in excretion of total purine nitrogen In mongrels 3 and 7 in which the

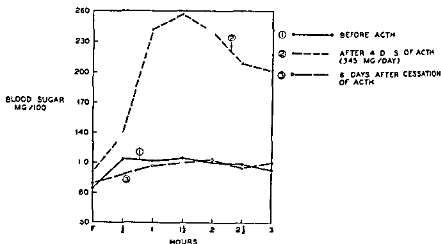


Fig 3 Effect of ACTH upon glucose tolerance Dalmatian 1

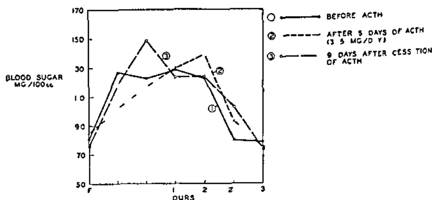


Fig 4 Effect of ACTH upon glucose tolerance Mongrel Dog 5

increases in uric acid excretion were twice that of mongrel 5 a moderately severe plateau type of curve was seen in one and a very mild impairment of glucose tolerance in the other Although the number of animals used does not allow a definite conclusion there appears to be a relation between the diabetic response and the increase of urinary uric acid excretion per kilogram of body weight

Negative nitrogen balance and increased renal excretion of 11 oxysteroids and 17 ketosteroids occurred during ACTH administration in both breeds There was no correlation between the percentage rise of 11 oxysteroid excretion of the dogs and the data previously discussed

A lowering of blood glutathione was observed only in Dalmatian 1

SUMMARY

Two Dalmatian and three mongrel dogs were given 345 mg of ACTH per day for periods of four to six days The more significant observations were as follows

1 The excretion of total purine nitrogen was increased by an average of 25% in both strains Uric acid constituted the major increment in the Dalmatians while it was predominantly allantoin which increased in the mongrels Elevated allantoin excretion persisted in the recovery periods for as long as 15 days

2 Increased production as well as increased clearance accounted for the increment in renal excretion of purines

3 Both groups exhibited negative nitrogen balance and increased excretion of 11 oxysteroids and 17 ketosteroids

4 Typical diabetic glucose tolerance curves were obtained with both Dalmatians and one mongrel dog A second mongrel exhibited a very mild decrease in carbohydrate tolerance while a third showed no change

5 There appears to be a relationship between the diabetic response and the increase of urinary uric acid excretion per kilogram of body weight

These observations suggest that the differences in susceptibility of dogs to the diabetogenic effects of ACTH are related to individual *variations in the response of purine metabolism to ACTH*

DISCUSSION

DR JOSEPH J BUNIM (Bellevue Hospital and New York University College of Medicine New York) Dr Bien and Dr Troll working with our group at the New York University College of Medicine observed that a given amount of uric acid will reduce arsenophosphotungstic acid in the presence of glucose to a greater extent than in the absence of glucose This introduces a considerable error when solutions analyzed for uric acid contain glucose This error increases as the amount of glucose increases so that a urine specimen containing for example 4 5% of glucose will introduce an error as great at 50% in the uric acid value as determined by the Folin method

Dr Milton Levy observed many years ago that if a borate buffer is introduced it will bind the glucose and prevent the reduction of arsenophosphotungstic acid In the uricase method a borate buffer is used but is routinely omitted from the blank If this precaution should not be followed an error would result even when the uricase method is used

This observation was made while studying a case of rheumatoid arthritis in a child of ten in whom diabetes was induced by cortisone The data which are pertinent to the subject of ACTH Induced Changes in Carbohydrate Metabolism will be presented

This child had a normal glucose tolerance test before the administration of cortisone There is no history of diabetes in the family After receiving 100 mg of cortisone daily for four days she developed severe glycosuria and mild hyperglycemia The glucose tolerance test done on the sixteenth day of cortisone therapy is shown in Figure 5

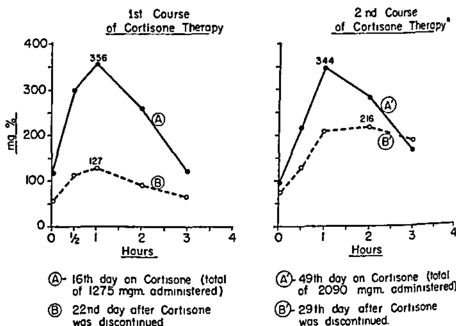


FIG 5 Effect of cortisone on glucose tolerance

(left) It will be seen that a peak of 365 mgs % was reached At that time she was excreting about 90 grams of glucose a day on a C 250 diet Three weeks after cortisone was discontinued the glucose tolerance curve returned to normal However when the patient was given the second course of cortisone (Figure 5 right) abnormal glucose tolerance again developed This time normal tolerance was not recovered until four weeks after cortisone was discontinued

(Figure 6) To date this child has been given cortisone over a period of sixteen months By reducing dosage marked clinical improvement was sustained without serious impairment in carbohydrate metabolism

DOCA was administered to determine whether it would antagonize the diabetogenic effect of cortisone It did not Later insulin was administered and it was observed that the child was not resistant to insulin since the urine was free of sugar when 50 units of crystalline insulin in three divided doses were given during the day

Another point of interest is that during the night from 8 p m to 8 a m she excreted about 40% of the sugar excreted in the entire 24 hour period The blank portion of the columns after April 13th indicates sugar excreted during the night and the solid area shows the day urine The fasting blood sugar in the morning was about 80

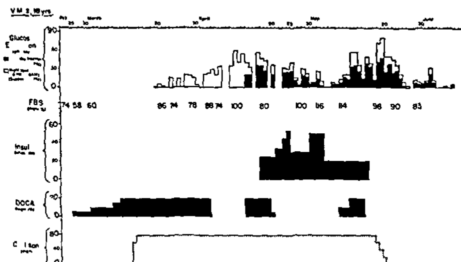


Fig 6 Rheumatoid arthritis

mgs % which leads us to suspect that there may have been a tendency toward renal glycosuria as well as diabetes mellitus Renal diabetes induced by ACTH has been reported by Kass Ingbar and Finland

After our patient had been treated with cortisone for twelve months a four hour test of 25 mgs of ACTH produced a satisfactory eosinopenia of 75%

DR WILLIAM Q WOLFSON Figure 7 is concerned with the effect of ACTH upon the rate of urate production in man Recent isotope studies have shown urate to be a true end product of nucleoprotein metabolism in man Apart from the 10% to 20% which has long been known to be lost into the gut as an entero hepatic component of urate excretion all of the urate formed is excreted into the urine (These figures apply only if renal function is normal with impaired renal function the entero hepatic component is much larger) In the study

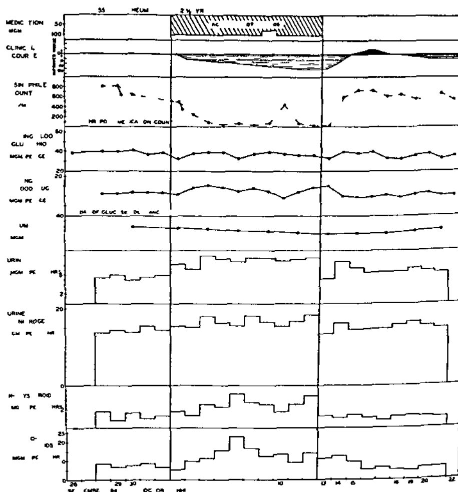


Fig 7 The scale for urine urate is given incorrectly values should be multiplied by 100

shown in Figure 1 daily urate excretion increased from an average of about 550 to about 800 mg /day during the administration of ACTH although plasma urate level did not change appreciably Since even diversion of the entire entero hepatic component of urate ex cretion to the renal pathway could not have produced such an in crease it is apparent that ACTH increased the rate of urate produc tion here as in Dr Fajan s dogs From these results recent studies suggesting that ACTH acts solely by increasing urate clearance in man appear to give an incomplete picture

With increasing evidence that an increased rate of urate produc tion is characteristic of gout one may ask why gout attacks should yield dramatically to an agent which increases urate production

There are several possible answers to this question, of which the most plausible is the old idea that the symptoms of gout are due not to increased urate production but to failure to dispose of some urate precursor sufficiently rapidly. Acute gouty arthritis, to our knowledge, has never been precipitated by injecting urate but has on a few occasions followed administration of adenine or adenosine. In last year's Conference we attempted to indicate that ACTH, even in large doses, modifies the disturbed purine metabolism in gout but does not induce normal purine metabolism.

Figure 8 is a preliminary attempt to explore the renal mechanism by which the increased urate excretion of the Dalmatian is accom

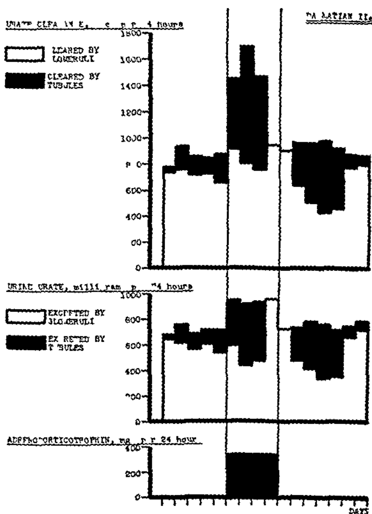


FIG 8

plished For this purpose Drs Fajans Conn and Johnson have kindly permitted recalculation of data obtained during their study on Dalmatian II Using allantoin clearance to estimate glomerular filtration rate these data confirm the reported tubular secretion of urate by the Dalmatian kidney

ACTH induced a marked increase in both the glomerular and tubular clearances of urate An apparent sudden interruption of tubular urate secretion which occurred on the final day of ACTH administration and the first post ACTH day may be a laboratory artifact dependent on a single high plasma urate value which could not be checked because of a small sample or it may indicate tubular injury due to excessive amounts of corticoid

When ACTH was withdrawn there was a marked time differential between glomerular and tubular components in their return to baseline values Glomerular urate clearance decreased rather promptly while tubular urate clearance continued to be elevated by some days after ACTH was withdrawn Although further studies are necessary to clarify the details of these changes these suggest that ACTH profoundly modifies the tubular transfer of urate in the Dalmatian dogs

In view of Dr Bunim s comment I should add that the amounts of glucose excreted in the studies summarized in Figures 7 and 8 were unimportant so far as any possible effect on urate values is concerned

DR STEFAN S FAJANS In reference to Dr Bunim s remarks I would like to mention that we have employed the modification of Bien and Troll in our determinations of urinary uric acid

PART IV

CARBOHYDRATE AND FAT METABOLISM

30

The Effect of ACTH on Glucose and Ketone Production in Phloridzinized Rats*†

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The glucoside phloridzin has long been recognized as an agent which will lower the renal threshold for glucose Therefore within limits glucose and nitrogen excretion in animals given phloridzin can be employed as an index of the capacity of such animals for gluconeogenesis Accordingly studies were undertaken for the standardization of the response of rats to phloridzin with a view to using this as a tool for the study of the effect of ACTH and adrenal steroids on gluconeogenesis

MATERIAL AND METHODS

The animals employed in this study were all young mature male rats of the inbred Fischer strain bred in our own laboratories After intact animals had fasted for 24 hours they were injected with an aqueous suspension of ACTH (6 animals for each dose) 1 cc of sesame oil and 50 mg phloridzin suspended in 1 cc of olive oil (In

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* This study was made possible by grants from Ayerst McKenna and Harrison Ltd the National Cancer Institute of the National Institutes of Health the Damon Runyon Memorial Fund and the American Cancer Society

† We would like to thank Dr E E Hays of Armour and Company for ACTH Dr F C Reifenstein Jr of Ayerst McKenna and Harrison Ltd for phloridzin and Dr H I Mason of the Mayo Foundation Dr W J Haines of The Upjohn Company Dr E Oppenheimer of Ciba Pharmaceutical Products Inc Dr G Pincus of the Worcester Foundation for Experimental Biology Dr P L Julian of The Glidden Company and Dr J M Carlisle of Merck and Company Inc for supplies of steroids

the adrenalectomized animals the sesame oil was the vehicle for the steroids) The animals were then placed in individual metabolic cages and urine was collected under mineral oil with sodium fluoride as a preservative Six hours later the animals were given the second injection of ACTH Fasting and urine collection were continued until 24 hours after the initial injection A group of 12 control animals received identical treatment except for ACTH injections

Another group of animals was adrenalectomized three days later after fasting for 24 hours injections with various steroids in 1 cc of sesame oil (at least 6 animals for each dose) and 50 mg of phloridzin in 1 cc of olive oil were given Fasting was continued and urine collected as in the previous group

Glucose concentration was determined by Benedict s quantitative method[1] nitrogen by semi micro Kjeldahl analysis and acetone by the colorimetric modification of the Van Slyke method described by Crandall[3] All values for excretion of glucose nitrogen and ketone were calculated as mg per 100 Gm rat

RESULTS

The 12 intact rats which were given no ACTH excreted an average of 668 mg glucose 215 mg of nitrogen and 31 4 mg of ketone per 100 Gm rat

Figure 1 compares the effects of varying amounts of ACTH on the phloridzinized rats The results are represented as per cent change from the values obtained in the intact controls Small amounts of ACTH (1 and 2 mg equivalent of Armour standard La 1 A) produced a decrease in the amount of nitrogen and glucose excreted As the dosage of ACTH was increased the amount of nitrogen excreted remained small while a substantial increase in glycosuria was noted The increase in glycosuria was accompanied by an even greater increase in ketonuria

In a search for an explanation of these results the effect of adrenalectomy was first studied Adrenalectomy lowered the urinary excretion of ketones to such an extent that in none of 13 animals was any ketone found by our method of analysis The glucose concentration dropped to 50 mg and the nitrogen to 16 6 mg per 100 Gm rat

The effects of various adrenal steroids were then studied in adrenalectomized animals Compound A acetate Compound F and Compound B (corticosterone) all produced similar results Increasing the amount of steroid used produced a sharp rise in urinary excretion of glucose Even the largest doses used in this study however failed to increase the excretion much higher than half the level attained by intact untreated animals The excretion of nitrogen rose *pari passu*

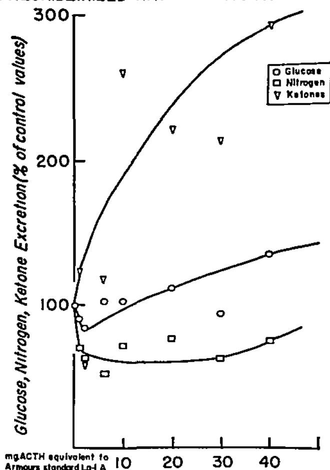


FIG 1

with that of glucose Of particular interest is the fact that with these three compounds it was possible to equal or exceed slightly the ketone excretion attained even by intact animals A whole hog adrenal extract was also studied and the excretion of glucose and nitrogen was found to be similar to that obtained with the three previously mentioned pure steroids Ketone excretion however reached a maximum at the dose equivalent to 1 mg of cortisone and decreased with larger doses even though glucose excretion continued to rise

Cortisone acetate and desoxycorticosterone acetate gave results which were not as good as those attained with the previously mentioned steroids The rise in glucose excretion was not as steep and

plateaued off at a level about one half that attained with the other steroids Only with extremely high doses was slight ketonuria obtained

Many other steroids were studied including Compound S acetate progesterone testosterone Δ 5 pregnenolone acetate and 21 acetoxy

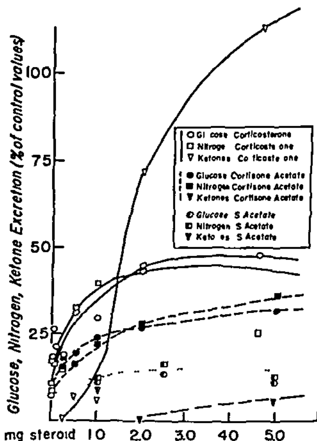


Fig 2

pregnenolone Compound S acetate was the most effective of this latter group although none of these produced more than a slight alteration in any of the excretions studied

Figure 2 compares the effects of three steroids corticosterone cortisone acetate and Compound S acetate each selected as representative of one of the three groups mentioned above The curves for corticosterone show a restoration of glucose and nitrogen excretion to

about one half that of intact animals and a complete restoration of ketonuria Curves for Compound F Compound A acetate and hog adrenal extract would follow a similar pattern Cortisone acetate illustrates the group of less active compounds which also includes desoxycorticosterone acetate Compound S acetate however was selected not as typical of the least active group but as the only compound in that group which produced any appreciable increase in the excretion values studied It should be noted that of the entire group of animals treated with the compounds of this last group only one animal (given 1 mg of Compound S acetate) excreted any ketones

GENERAL DISCUSSION

From the data presented it appears that ACTH is capable of substantially increasing the glycosuria in phloridzinized intact rats and that this increase in glucose is accompanied by an increase not in urinary nitrogen but in urinary ketones Since a 24 hour fast will deplete the liver glycogen it is doubtful that this non nitrogenous source could be the origin of the carbohydrate Thus it seems that body fat is the probable source of the increased glucose The accompanying great increase in urinary ketones would support this theory

The studies with Compounds A B and F further substantiate this view since with these there also was an increase in excretion of glucose and nitrogen accompanied at higher dosages of steroid by a disproportionately great increase in ketones These observations would argue in favor of a role for the adrenal steroids in the break down of fat

The minor differences between some of the steroid effects reported in this paper and those obtained by Wells and Kendall[4,5] are probably due to experimental design and animal material

In an evaluation of the effects of ACTH it should be realized that the fasted intact animal given phloridzin is probably in an acute condition of stress and producing carbohydrate from nitrogenous sources possibly at a maximal rate Administration of small amounts of ACTH to such an animal apparently changes the secretion pattern of the adrenal cortex disadvantageously and produces the observed decrease in glucose and nitrogen production However since the steroids produced under this new pattern are in all likelihood those found to have a substantial effect on ketonuria the ketones were increased during this same stage With further increases in the dose of ACTH the excretion of glucose then became greater than that observed in the intact animal and was accompanied by a further increase in ketonuria

SUMMARY

ACTH was given to intact rats which were also treated with phloridzin This led to greatly increased glycosuria unaccompanied by an increase in urinary nitrogen but accompanied by an increase in urinary ketones

Similar studies in adrenalectomized animals employing the various active steroids from the adrenal cortex showed that with certain of these steroids there is an increase in excretion of glucose and nitrogen with an associated increase in ketonuria

These facts are interpreted as indicating that under the influence of adrenal steroids either directly or through their increased production as brought forth by ACTH the rat is capable of making glucose for energy requirements from fat as well as from protein

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DISCUSSION

DR LAURANCE W KINSELL Just a brief comment We will show in the next two papers that in the human subject the administration of ACTH actually decreases the level of blood ketones

We think that whatever other comments one might make it is well to recall that there are multiple differences between rodents and humans Nonetheless we will agree with Dr Segaloff in that we believe that the evidence in both humans and rats is strongly in favor of neoglucogenesis from fat in response to ACTH administration

DR GEORGE W THORN There has been no mention made of renal threshold I should point out that the older studies of Drs Drury McKay and others suggest that in the adrenalectomized animal the renal threshold for ketone bodies may be altered Thus one possible interpretation of these studies may involve a change in the renal threshold of ketone bodies

DR JEROME W CONN I should like to make one short comment in support of Dr Kinsell s remark namely that there appears to be a difference between man and animals with respect to the ketogenic action of these steroids

Many of you recall Dr Sprague s experiments with cortisone in the Addisonian who also has diabetes He observed a prompt increase in ketonemia and ketonuria

Our experiments with Compound B however in a similar preparation Addison s disease plus diabetes fail to show any increase in ketones while these experiments of Dr Segaloff in the rat show a sharp increase in ketosis with Compound B

DR PETER H FORSHAM We have used patients with Addison s disease as experimental subjects Dr L Bennett of the University of California showed I think without much doubt that there was an increase in ketone bodies when such patients were given cortisone after an initial fast However this increased ketonemia did not persist for more than three to four days In other words at first the results agreed with those of Dr Segaloff and ended up in the other camp

Our explanation was that if you give relatively little hormone which Dr Segaloff did also when he started out you will initially be able to show an increased ketone body production as the liver glycogen is low but as the glycogenic effect of these adrenal agents increases this ketogenic effect will be blotted out

DR ALBERT SEGALOFF With reference to Dr Forsham s remarks I might point out that a phloridzinized animal is without a renal threshold for glucose and cannot produce glycogen so you would expect it would go along as his patient who had no glycogen stores

SUMMARY

ACTH was given to intact rats which were also treated with phlorizin This led to greatly increased glycosuria unaccompanied by an increase in urinary nitrogen but accompanied by an increase in urinary ketones

Similar studies in adrenalectomized animals employing the various active steroids from the adrenal cortex showed that with certain of these steroids there is an increase in excretion of glucose and nitrogen with an associated increase in ketonuria

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tion of ACTH in a dosage of 100 mgm daily It will be noted that the average elevation of total blood ketones in this group of patients by the end of the third day of the control fast reached a level of 27 mgm % as compared to a prefast level of less than 2 mgm % The same individuals when treated with ACTH during the course of the fast had average blood ketone levels at the end of the third day of the fast of less than 7 mgm/100 cc of blood

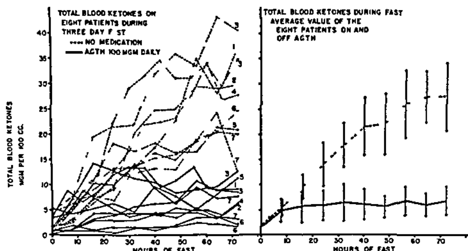


Fig 1 Individual and average blood ketone values obtained in twenty fasting studies in eight representative individuals The broken lines represent the values obtained during the control fasts the solid lines represent blood ketones during fast plus ACTH administration

EFFECT OF ACTH AND OF CORTISONE UPON FASTING INDUCED HYPERKETONEMIA IN NON DIABETICS

In Figure 2 is shown a series of five fasts in a young male arthritic patient The findings in this man are characteristic of more than 90% of all patients so far studied The pertinent findings appear to be as follows

1 During the initial fast there is a progressive rise in blood ketones (as well as in urinary ketones) a progressive fall in blood sugar and a tendency to fall in total eosinophils

2 In the course of the second fast during which time the patient receives ACTH there is a much lesser rise in blood and urinary ketones a rise rather than a fall of blood sugar essentially complete disappearance of circulating eosinophils and a slightly greater output of urinary nitrogen

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Hormonal Regulation of Fat Metabolism I Effects of ACTH and of Certain Steroid Hormones upon Fasting Induced Hyperketonemia

Laurance W Kinsell, Sheldon Margen, George D Michaels and John Partridge

with the technical assistance of Gerald Liebert Carl T Anderson and Judith Lange

INSTITUTE FOR METABOLIC RESEARCH OF THE HIGHLAND ALAMEDA COUNTY HOSPITAL OAKLAND CALIFORNIA

Preliminary reports from this laboratory have indicated that certain steroid hormones including testosterone exert a significant effect upon fat metabolism [1]

To further evaluate the effects of various hormonal agents upon fat metabolism a standard fasting procedure was devised During the period of fast (three and one half days) the individual receives only distilled water and any specific medication which is under evaluation Prior to during and following the fast a large number of blood and urinary constituents are measured at frequent intervals For the purpose of this presentation emphasis will be placed only upon blood and urinary ketones blood and urinary sugar urinary nitrogen circulating eosinophils and in some instances urinary 17 ketosteroids Each individual acts as his own control that is to say during the control fast the patient receives no treatment of any sort subsequent fasts are carried out in an identical fashion excepting only that the patient receives a specific hormonal agent

More than fifty individuals so far have been studied in this fashion In some of them as many as nine fasts have been carried out with suitable intervals intervening between fasts to enable the individual to return to his basal state Included among the individuals so studied have been relatively normal adults patients with rheumatoid arthritis leukemia diabetes and a considerable variety of other medical abnormalities

In Figure 1 are shown the results of twenty such fasting studies carried out in eight representative individuals prior to the administration of any therapy and then subsequently during the administra

EFFECT OF ACTH UPON FASTING INDUCED HYPERKETONEMIA IN A PATIENT WITH MILD DIABETES MELLITUS

It was anticipated that fasting would induce a greater degree of hyperketonemia in a diabetic than in a normal individual The initial evaluation of this hypothesis was carried out in a middle aged male diabetic whose diabetes was not well controlled and who at the time of the study was receiving no insulin The findings in this patient are shown in Figure 3 The following data were obtained

1 Testosterone propionate did not significantly modify the fasting induced hyperketonemia It appeared however to significantly diminish the pre fasting level of blood ketones which had been hyper normal in this patient

2 The administration of ACTH resulted in a significant diminution in the fasting induced hyperketonemia at the same time that some elevation of blood sugar and some increase in urinary nitrogen occurred

3 A fast carried out during the post testosterone rebound period resulted in complete suppression of hyperketonemia

EFFECT OF ACTH UPON FASTING INDUCED HYPERKETONEMIA IN A PATIENT WITH SEVERE DIABETES MELLITUS

Figure 4 presents the effect of fasting and of fasting plus ACTH upon the blood and urinary sugar and ketones in an individual with moderately severe diabetes The patient is a young girl of 20 whose insulin requirement is approximately 80 units per day It will be noted that during the control fast a major elevation of blood ketones occurred on the third day coincident with a progressive rise in blood sugar The second fast during which the patient received ACTH had to be terminated on the second day because of progressive dehydration and acidosis The interpretation of these findings will be considered below

EFFECT OF ACTH UPON FASTING INDUCED HYPERKETONEMIA IN A PATIENT WITH INSULIN RESISTANT DIABETES

Figure 5 shows the effect of fasting and of fasting plus ACTH upon the blood ketones of a young woman with an extremely insulin

3 In the course of the third fast which occurs after the patient has been on ACTH for a much longer period of time there is only minimal elevation of blood and urinary ketones in the course of the fast and a slightly greater output of urinary nitrogen In other respects the findings are identical with fast number two

4 Fast number four begins two days after administration of

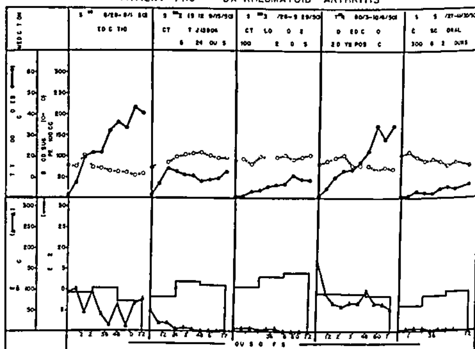


FIG 2 Serial fasting studies in arthritic patient Fro It will be noted that ACTH and 11 dehydro 17 hydroxy corticosterone orally administered produce identical effects upon blood ketones (In a patient with acromegaly increased elevation of blood ketones was noted during compound E administration whereas ACTH produced its usual effect)

ACTH has been stopped The findings are almost identical with control fast number one

5 Fast number five is carried out during the administration of 300 mg of cortisone daily administered orally The effect upon blood ketones is identical with that noted for ACTH and the same statement applies to most of the other findings with the exception of blood sugar which falls slightly instead of rising during the course of the cortisone administration

EFFECT OF ACTH UPON FASTING INDUCED HYPERKETONEMIA IN A PATIENT WITH MILD DIABETES MELLITUS

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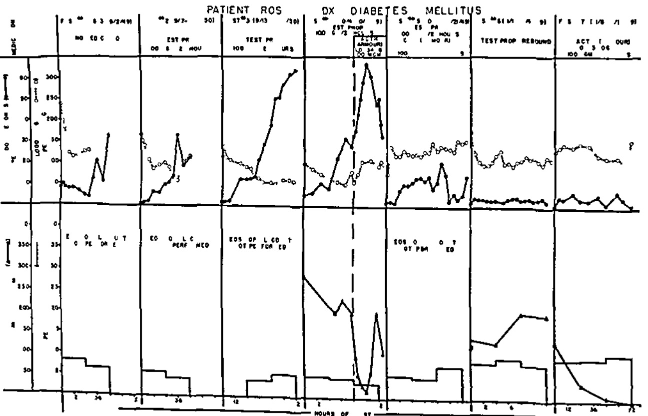


FIG 3 Effects of testosterone of ACTH and of post testosterone rebound upon a patient with mild diabetes mellitus Fasts #1 and #2 extended for only two days

resistant form of diabetes associated with hirsutism and other evidences of endocrine dysfunction * This patient on one occasion *had received in excess of 20 000 units of insulin in a single day without evidence of any effect upon her blood sugar* Another interesting finding in this girl was the observation that despite the loss of a great

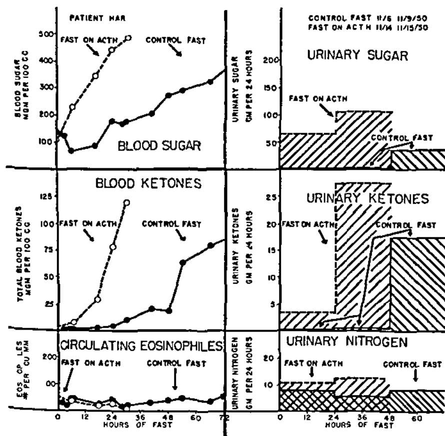


FIG 4 Effects of ACTH upon carbohydrate and ketone metabolism in a severe diabetic The hyperketonemia which occurred *during* ACTH administration may be referable to vastly increased fat combustion *as the result of inability to oxidize newly formed carbohydrate*

amount of sugar daily in her urine and the occurrence of a fasting blood sugar well in excess of 300 mgm/100 cc ketones had never been found in her urine

It will be noted that during the control fast only minimal elevation of blood and urinary ketones occurred In the course of fast number two during which the patient received ACTH the blood and

* We are indebted to Dr Myron Arrick for the opportunity of studying this patient

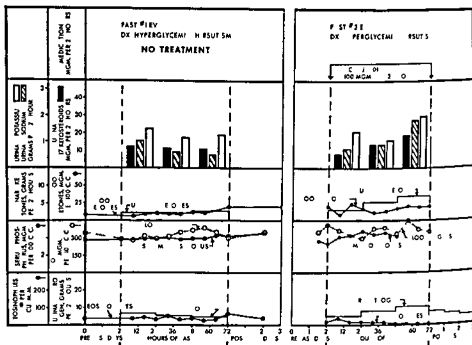


FIG 5 Fasting studies in a patient with extreme insulin resistance as sociated with other evidence of endocrine dysfunction It will be noted that relatively slight elevation of blood ketones occurred during the con trol fast.

urinary ketones if anything were higher than had been the case dur ing the course of the initial fast The urinary nitrogen excretion was moderately higher during the second fast and the blood sugar levels were also slightly higher

EFFECT OF ACTH UPON FASTING INDUCED HYPERKETONEMIA IN AN ACTH RESISTANT ARTHRITIC

In Figure 6 are shown the findings in a young male arthritic pa tient This man failed to make an adequate clinical response to large dosage of ACTH and after an initial brief fall of eosinophils failed to manifest any eosinopenia There was however a very significant increase in adrenocortical activity in terms of a major elevation in urinary 17 ketosteroids It will be noted that this patient failed to have any suppression of his fasting induced hyperketonemia during ACTH administration in fact the levels of blood and urinary ke tones during fast number two and number three (on ACTH) were higher than those noted during the control fast

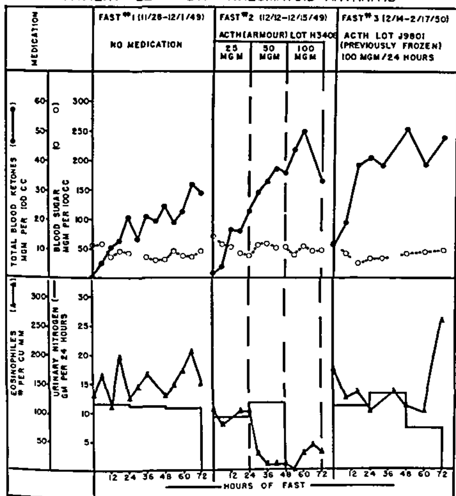


Fig 6 Lack of suppression of fasting induced hyperketonemia by ACTH in an arthritic patient who also failed to make an adequate clinical response to ACTH It will be noted that major elevation of urinary 17 ketosteroids occurred

GENERAL DISCUSSION

The administration of ACTH and of cortisone to a majority of normal and abnormal individuals results in diminution or obliteration of fasting induced hyperketonemia and ketonuria Certain possible interpretations present themselves in terms of mechanism

1 That some adrenal steroid or steroids greatly increase the rate

of utilization of ketones that is to say they stimulate the rate of ketolysis

2 That certain adrenal steroid hormones change the catabolic pathway of fatty acids in such a way as to decrease or eliminate the formation of ketones as intermediary metabolites

3 That some adrenal steroids decrease the total energy requirement in the fasting individual and hence diminish the demand for fat combustion Brief respiratory quotient studies which have been carried out in some of these patients show no evidence of decrease in oxygen requirement so this hypothesis appears untenable

4 That certain adrenal steroid hormones increase the total oxidative catabolism of amino acids to a degree sufficient to exert a major fat sparing action This hypothesis also appears to be untenable in view of the relatively slight increase in nitrogen excretion which occurs in the course of fast during ACTH administration as compared to the control fast

It appears then that the diminution in fasting induced hyperketonemia in response to ACTH administration is referable to acceleration of ketolysis and/or to major modification of the catabolic pathway of fat If the latter mechanism is responsible the possibility of neoglucogenesis from fat could be considered The marked increase in blood and urinary sugar completely out of proportion to any increase in urinary nitrogen in diabetic patient Har (Figure 4) during ACTH administration (fast number two) would support such a concept Further studies in this and other diabetic patients including simultaneous respiratory quotient determinations will be reported elsewhere

The findings in the insulin resistant diabetic (Figure 5) deserve some comment This patient (whose general endocrine picture was compatible with some type of hyperadrenocorticism) *apparently* produced one or more endogenous steroids which produced much the same effect upon fat catabolism as that which one observes in response to ACTH administration

Patient Lew (Figure 6) presents findings which are capable of at least tentative interpretation He failed to make an adequate response to ACTH *in terms of clinical improvement* fall in eosinophiles or suppression of fasting induced hyperketonemia Nevertheless he manifested a major response in terms of increase in urinary 17 ketosteroids One might therefore assume that this man made an abnormal qualitative adrenal cortical response to ACTH

In the paper which follows Dr Margen will present the results of *infusion* studies using aceto acetic acid and octonoic acid which may help to clarify the mechanism of ACTH and cortisone effect upon fat metabolism

SUMMARY

The administration of ACTH and of cortisone to individuals on a standard three and one half day fast results in major diminution or suppression of the fasting induced hyperketonemia The possible mechanisms of action are discussed

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DISCUSSION

Discussion follows next paper

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Hormonal Regulation of Fat Metabolism II Effects of ACTH and Certain Steroid Hormones upon the Utilization of Infused Aceto Acetate and Octonoic Acid

Sheldon Margen George D Michaels Lenore A Boling and Laurance W Kinsell

with the technical assistance of Ge ald L ebert Judith Lange and Carl T Anderson

INSTITUTE FOR METABOLIC RESEARCH HIGHLAND ALAMEDA COUNTY HOSPITAL, OAKLAND CALIFORNIA

It has been shown in the previous paper that ACTH and cortisone when administered in adequate dosage decrease or eliminate fasting induced hyperketonemia The mechanism of this effect appears to be acceleration of the rate of peripheral ketolysis and/or change of the catabolic pathway of fatty acids in such a fashion as to decrease or eliminate the formation of ketone bodies

Two techniques have been devised to further evaluate the above mechanisms *The first consists of the infusion of 200 mgm of sodium* acetoacetate per kilogram of body weight (as a 4% solution expressed as acetone) over a thirty minute period Blood and urine ketones and blood sugar are quantitated at suitable intervals preceding during and following the infusion *The second* consists of the infusion of 200 mgm of octonoic acid per kilogram of body weight (administered as a solution of sodium octonoate equivalent to 2% octonoic acid) over a 60 minute period Blood and urine octonoate ketones and sugar are determined at suitable intervals preceding during and following the infusion As in the case of the standard fasting procedures each individual acts as his own control i e control infusions are carried out followed by infusions during treatment with the specific hormonal agents

RESULTS

Acetoacetate Infusion

In Figure 1 are shown the results of two control infusions in a normal young adult male It is apparent that reproducible curves can

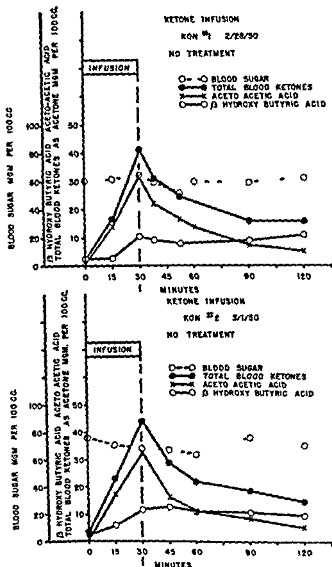


FIG 1 Control sodium acetoacetate infusions in a normal adult male

be obtained at least in a given individual This statement applies to total ketones and also to partition between acetoacetic and β hydroxybutyric acid

In Figure 2 are shown the results of an infusion in an individual during the period of ACTH administration and a subsequent infusion during cortisone administration The rate of disappearance of total ketones on the second day of ACTH administration differed only slightly from the control studies On the third day of ACTH ad

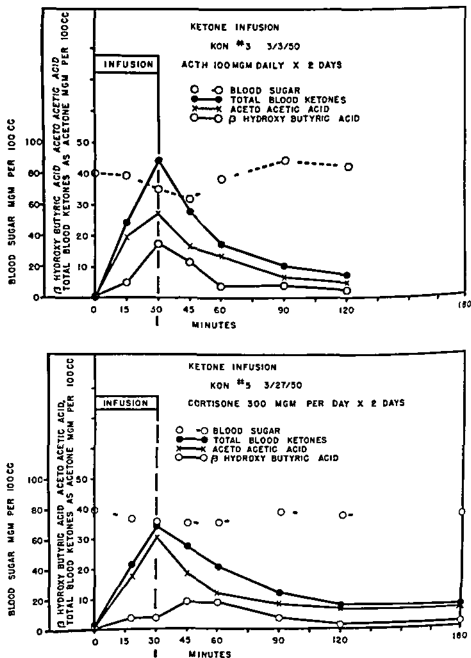


FIG 2 Sodium acetoacetate infusions in a normal adult male during ACTH and cortisone administration

ministration however the maximal level of blood ketones was significantly lower (at the end of the infusion) as were also the blood levels after the infusion had been completed These results were

easily repeated with comparable findings on a number of occasions

Similar studies carried out in a middle aged diabetic patient failed to reveal any evidence of acceleration of ketolysis although the diabetogenic effect of the material is apparent (Figures 3 4 and 5)

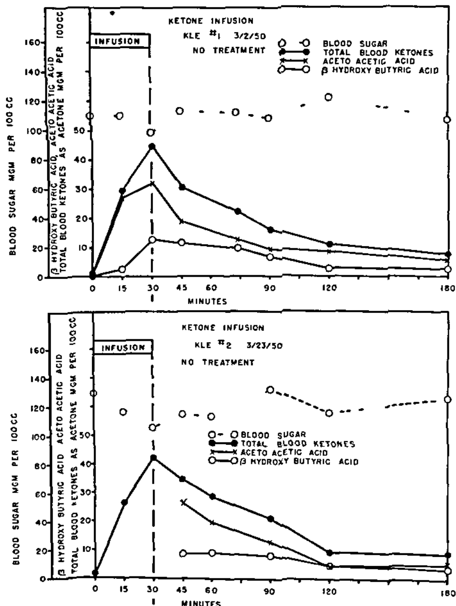


FIG 3 Control sodium acetoacetate infusions in a mild diabetic

HORMONAL REGULATION OF FAT METABOLISM II

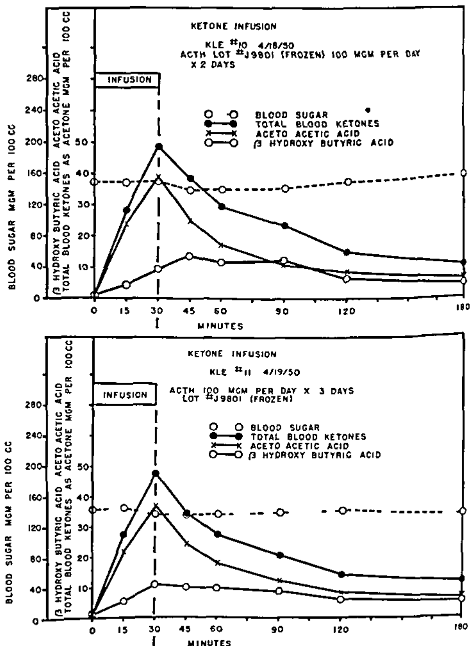


Fig 4 Sodium acetoacetate infusions in a mild diabetic during ACTH administration

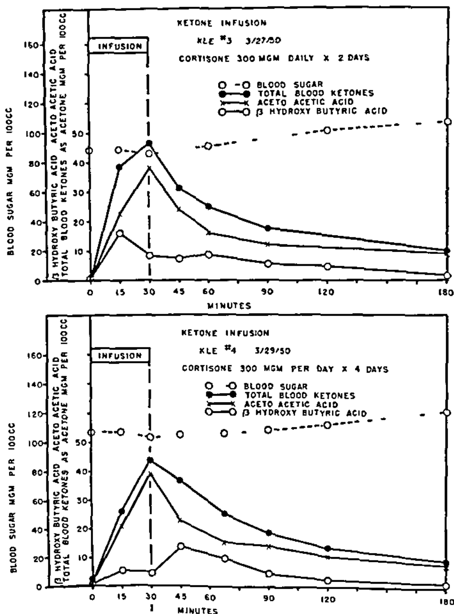


FIG 5 Sodium acetoacetate infusions in a mild diabetic during cortisone administration

Octonoate Infusion

During the first several months of experimentation with this technique a thirty minute infusion period was used It was found that the amount of octonoate which could be administered over such a period was not sufficient to permit of the demonstration of significant effects of therapy (The toxicity of octonoic acid is such as to limit the amount which can be administered in a given period of time) Consequently only a limited amount of data are as yet available using the 60 minute infusion procedure Figure 6 shows the blood octonoate and ketone levels obtained in a young male patient suffering from rheumatoid arthritis The control values and those obtained during ACTH therapy each represent the average of two infusions It appears that ACTH decreases the maximal elevation of blood octonoate and also of blood ketones formed from octonoate

No octonoate was found in the urine at any time

GENERAL DISCUSSION

The purpose of the studies described in this paper is to determine whether ACTH and steroids resulting from ACTH administration increase the rate of peripheral ketolysis or change the metabolic pathway of fatty acids It seemed reasonable to believe that if the former were the case infused acetoacetate would disappear more rapidly from the blood during ACTH administration If the ACTH effect were one of alteration of the metabolic pathway of fatty acids infused fatty acid should disappear more rapidly and ketone formation from such infused material should be diminished

From the data so far available one may tentatively conclude that both effects occur There appears to be no question that in patient FRO (Figure 6) increased metabolism of fatty acid without increased ketone formation occurs In patient KON (Figures 1 and 2) the data suggest increased utilization of infused ketones The reason for the lack of such an effect in diabetic patient KLE (Figures 3 4 and 5) during ACTH administration is not immediately apparent

Further studies of this sort are at present in progress including prolonged fatty acid infusions in association with continuous respiratory quotient determinations

SUMMARY

Two techniques have been devised to further evaluate the mechanism of the effect of ACTH and adrenal steroids upon fat metabo

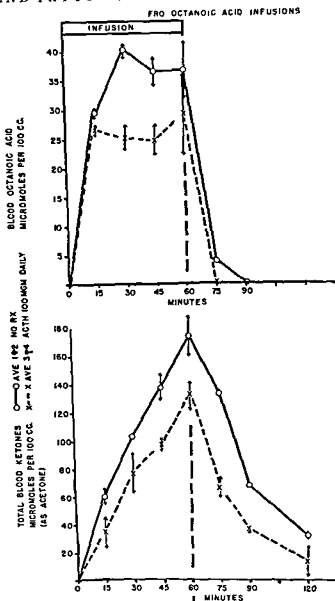


Fig 6 Comparative blood octonoate and ketone values in patient FRO (control infusions and infusions during ACTH administration)

lism The first consists of the infusion of acetoacetate and the second of the infusion of octonoate under standard conditions

From the data so far obtained it appears that adrenal steroids resulting from ACTH administration may both accelerate the rate of ketolysis and also modify the catabolic pathway of fatty acids

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DISCUSSION

DR STEFAN S FAJANS Figure 7 presents observations made on a 28 year old female with severe juvenile diabetes mellitus This patient received a total of 2 825 mg of ACTH in the course of 22 days in the hope of influencing a recently developed subacute myelogenous leukemia

The patient was fed a constant diet A total of 72–76 units of insulin was given daily throughout the study with exceptions as noted on the slide

During the baseline period glycosuria varied between 26 and 91 grams per day Insulin sensitivity was observed in the control period inasmuch as there was a precipitous drop in the blood sugar between 8 a m and 12 noon on the third and fourth day

During ACTH administration there was a considerable increase in glucose excretion which exceeded the dietary intake of carbohydrate on several days Thus in addition to the dietary carbohydrate a considerable amount of the glucose derived from gluconeogenesis was lost in the urine An insulin tolerance test was performed the morning after cessation of ACTH administration and showed almost complete resistance to insulin

Although it is not shown in this figure the patient was also in tremendous negative nitrogen balance during the latter part of the study On the last day of ACTH administration the urinary nitrogen excretion was 38 grams

Ketonuria was absent or minimal throughout the period of study The slide shows that although mild ketonemia existed baseline values were never exceeded during ACTH administration

The absence of significant ketosis during ACTH therapy was re

markable in view of the metabolic state of severe uncontrolled diabetes manifested by glucose excretion in excess of dietary intake of carbohydrate tremendous negative nitrogen balance and insulin resistance

DR JOHN E HOWARD (Johns Hopkins Hospital and Johns Hopkins University School of Medicine Baltimore) Relevant to Dr Kinsell s remarks are perhaps certain recent experiences at the Johns Hopkins

FIG 7 Effect of ACTH on fasting blood sugar glycosuria and blood ketones

Hospital on the effects of ACTH in a patient with insulin resistance A 63 year old man who had suffered with chronic lymphatic leukemia for four years restrained the lymphocyte count to the neighborhood of 50 000 with courses of splenic x ray

Diabetes was discovered in 1944 and had been readily controlled by diet alone until August 1949 when insulin therapy was begun In January 1950 weight loss and glycosuria occurred despite steadily increasing insulin dosage For 12 days in hospital 250–500 units of insulin per day did not completely combat the diabetes but gradually there was spontaneous remission of the resistant state and the patient was discharged regulated with 60 units per day

In June he was readmitted to the hospital for recurrent insulin resistance which had developed gradually over a three week period On one occasion insulin was tried intravenously (200 units in 400 cc saline) After 100 cc of this solution had run in slowly the patient suddenly collapsed became pulseless and unconscious With supportive measures he regained consciousness and blood pressure became obtainable in about 15 minutes but he was weak and lethargic for several days thereafter before regaining his previous status The reaction was interpreted as anaphyllactoid shock On several occasions (by no means all) during subsequent days indurated areas surrounded by urticarial wheals and erythema were noted at the sites of insulin injection A skin test was performed in which the patient reacted with vigorous wheal and flare to crystalline zinc insulin and with moderate wheal and flare to protamine zinc insulin A control patient gave no reaction to similar intradermal injections of these substances

Despite daily doses of insulin between 500 and 1000 units the patient developed severe acidosis from which he was (literally) rescued by intravenously administered sodium bicarbonate The patient was carried along only by the use of 3000 to 6000 units daily until during August for 28 days he received 50 mg ACTH per day in divided doses On the fifth day of ACTH hypoglycemic attacks caused sharp lowering of insulin dosage and for the subsequent three weeks his blood sugar followed a hectic curve between hyper and hypoglycemia but with in general greatly reduced insulin ration varying between none and 500 units Evaluation of his status during this period is difficult partly because of poorly recorded observations but there is little doubt that improvement in diabetic manageability and general well being of the patient occurred while ACTH was given

Within a few days of stopping ACTH insulin requirement again rose and he was given daily doses varying between 1000 and 3000 units Despite such doses hyperglycemia of 350 to 600 and glucosuria 50–100 grams persisted the difference in blood sugar levels and glucosuria seemingly but little altered whether 1000 or 3000 units were administered Despite this the patient maintained his weight did not vomit and though weak did not suffer serious complaints There was no acidosis

It was decided to try ACTH again but in order to control the experiment the patient was given a fixed diet and the insulin ration was reduced to a constant dose of 600 units per day On this regimen (see Figure 8) prior to ACTH in five days glycosuria and hyperglycemia increased and the patient lost 8 lbs but no ketonuria or acidosis appeared On September 23rd ACTH was begun the above regimen being continued Weight loss promptly ceased and the patient imme

diately began to feel better On the fifth day of 100 mg ACTH his blood sugar began to fall and glycosuria to lessen By the eighth day insulin was reduced to 100 units because of normoglycemia and on the 11th day insulin was withdrawn altogether ACTH was continued at 100 mg per day for 10 more days until October 13th when it was abruptly stopped During this period with ACTH but no insulin hyperglycemia and glycosuria returned in moderate degree (average 300 mg and 60 gms per day respectively) but the patient continued to feel well and weight loss was minimal

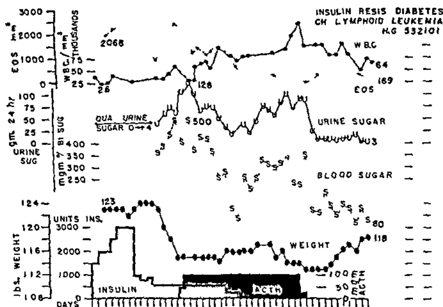


FIG 8

On withdrawal of ACTH fasting blood sugar promptly fell to levels ranging between 125 and 80 mg daily glycosuria did not exceed 5 grams and the body weight rose promptly On September 24th 10 days after ACTH was stopped the patient was discharged from the hospital in this situation without insulin and on the same diet Figure 8 depicts the course described above

At home he has done well spilling small amounts of sugar only when breaking his diet and periodic determinations of fasting blood sugar have all been within normal range

On November 12th a test period of five days with 100 mg ACTH per day (given in three doses intramuscularly at 8 hour intervals) was begun On the fourth day fasting blood sugar had risen to 200 mg and on the third fourth and fifth days glycosuria amounted to 14

In June he was readmitted to the hospital for recurrent insulin resistance which had developed gradually over a three week period On one occasion insulin was tried intravenously (200 units in 400 cc saline) After 100 cc of this solution had run in slowly the patient suddenly collapsed became pulseless and unconscious With supportive measures he regained consciousness and blood pressure became obtainable in about 15 minutes but he was weak and lethargic for several days thereafter before regaining his previous status The reaction was interpreted as anaphyllactoid shock On several occasions (by no means all) during subsequent days indurated areas surrounded by urticarial wheals and erythema were noted at the sites of insulin injection A skin test was performed in which the patient reacted with vigorous wheal and flare to crystalline zinc insulin and with moderate wheal and flare to protamine zinc insulin A control patient gave no reaction to similar intradermal injections of these substances

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Studies are now being carried out by Drs G S Mirick E C Brown and J S Murphy on sera obtained from this patient at various stages of the illness described above It is hoped that such studies may shed light on some of the hypotheses of the mechanism of action of ACTH in the clinical improvement noted

DR RULON W RAWSON Recently we have seen a similar problem to that described by Dr Howard A patient with a mild diabetes requiring 50 units a day of insulin while receiving cortisone in treatment of a renal carcinoma developed an insulin resistance and required between 700 and 800 units of insulin a day to control his diabetes

After several weeks during which he required this large dose of insulin HN was administered in an attempt to alter the growth of his tumor Within 48 hours after administering the nitrogen mustard the insulin requirement fell to the previous level of 50 units per day

DR GEORGE W THORN Did you make any studies of changes in respiratory quotient in your patients in conjunction with the ketone studies?

DR LAURANCE W KINSELL In reply to Dr Thorn s question we have some sporadic respiratory quotient determinations which are at least compatible with the concept of neoglucogenesis from fat On the immediate agenda are long term respiratory quotient determinations during fat feeding and fatty acid infusion procedures

DR SHELDON MARGEN In further answer to Dr Thorn s question—respiratory quotient data enable one to draw conclusions concerning the formation of carbohydrate from fatty acids only if the newly formed carbohydrate is stored or excreted Otherwise one notes only an R Q which is characteristic of fat combustion

grams for each day and weight remained stationary Within two days after cessation of ACTH the fasting sugar was again normal where it has remained to date and there has been no subsequent glycosuria

Comment

Previous experience with administration of ACTH and cortisone to diabetic patients had shown that hyperglycemia and glucosuria are increased (our own experience and that reported by Lukens[1]) but Kinsell[2] has noted that ketonuria and ketonemia are suppressed by ACTH in normals during fasting and in diabetic patients during insulin withdrawal A beneficial effect in our patient s carbohydrate utilization could thus hardly be expected from the usual effects of ACTH in the diabetic Our only hope was that ACTH might beneficially affect the state of insulin resistance by alteration of the hypersensitivity to insulin which seemed clearly to exist as judged by the anaphyllactoid reaction to intravenous injection the skin tests and local responses at certain insulin injection sites There has been as yet no light thrown on the mechanism whereby ACTH and cortisone reduce and allay certain manifestations of antigen antibody hypersensitivity Mirick[3] reviewed the experimental literature and together with his own clinical observations concluded that there is no evidence that ACTH and cortisone reduce antibody formation when there is persisting antigenic stimulus He did suggest however that there was evidence that ACTH reduces circulating antibody titre when such persisting antigenic stimulus is not present (as in case of patients previously immunized with typhoid vaccine)

Our patient had been receiving insulin in doses up to 3000 units per day which would be viewed as a persisting antigenic stimulus to antibody formation against insulin One would think that even the 600 units of insulin per day given during the first part of the ACTH course would also act in a similar manner Yet in both the first ACTH course with 50 mg q d and in the second course with 100 mg q d it seems clear that the patient s carbohydrate metabolism benefited greatly Clearly the original severe insulin resistance was overcome but it is also of great interest that when insulin was withdrawn but ACTH continued the patient reacted with hyperglycemia and glucosuria in just about the same degree one would expect from other mild diabetics to whom we have given ACTH It is difficult to escape the conclusion that the adrenal steroids secreted under the stimulus of ACTH were exhibiting two distinct effects one in overcoming a hypersensitivity to injected insulin the other in effecting the usual diabetogenic action antagonistic to the pharmacologic action of insulin

As to the former action either circulating antibody to insulin was suppressed (which seems unlikely in view of Mirick s observations quoted above) or despite maintenance of such circulating antibodies to insulin the adrenal steroid had either prevented antibody antigen combination or even after combination had effected some mechanism whereby insulin could gain access to the cells and carry on its normal function On the basis of present information the latter possibility seems more likely and may offer another clue in the complex problem of how adrenal steroids block hypersensitive phenomena

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Serum Lipid Analysis in the Nephrotic Syndrome under ACTH Administration*

J W Soshea and Edith B Farnsworth

NORTHWESTERN UNIVERSITY MEDICAL SCHOOL CHICAGO

We have found a marked decrease in serum cholesterol in patients with the nephrotic syndrome under treatment with adrenocorticotropin (ACTH Armour) Reports on the effect of ACTH in normal individuals with respect to serum lipids have indicated variable and on the whole moderate changes[1 2 3] The present analysis of lipid fractions was therefore set up with the following purposes (1) to obtain data relative to the sources of hyperlipemia in the nephrotic syndrome (2) to employ the nephrotic hyperlipemia to obtain information on the effect of ACTH on fat metabolism

TECHNICAL METHODS

In general the method of differential lipid assay was that employed by Popjak[4] The sera were extracted by the technic of Bloor[5] the phospholipids present in the redissolved residue were precipitated with acetone and magnesium chloride and analyzed by a modification of the method of Fiske and SubbaRow[6] In the supernatant total and free cholesterol are determined by a modification of the Schoenheimer Sperry procedure as proposed by Clarke and Marney[7] The same phospholipid free supernatant is used for triglyceride and cholesterol ester assay after conversion to the chromogenic hydroxamic acid derivatives of the fatty acids[8] Thus free and total cholesterol phospholipids and non phospholipid fatty acids are determined directly Total serum lipids cholesterol ester fatty acids and triglycerides are calculated from these data

This method of aggregate cholorimetric analysis favors the admission of any error into the systematic class an argument originally proposed by Boyd in relation to his aggregate dichromate oxidation method[9]

* This study was aided by a grant from the U S Public Health Service

CLINICAL MATERIAL AND RESULTS

Six patients ranging in age from 3 yrs to 58 yrs who typify the nephrotic syndrome constituted the series All subjects were placed on a diet containing 700 mgs of sodium Case #1 T K 3 year old male (Table I) whose disease was of 2 years duration showed the most profound changes in serum lipids Control determinations showed in mg% free cholesterol 421 total cholesterol 1291 cholesterol ester fatty acids 626 phospholipids as lecithin 702 non phospholipid fatty acids 4920 triglycerides 4510 and total serum lipids 7129 In 3 weeks the triglycerides dropped to 34 7 mg% Phospho

Table I

T K MALE 3 YRS

Date	*Rx*	*Chol terol*			*Phos phol p d (Leci thin)*	*Nonphos phol pid EFA*	*Trigly erides*	*T tal Lip d*
		F ee	*T tal*	*E ter FA*				
Control		421	1291	626	70?	49 0	4510	7129
Oct. 30	ACTH 12 mg							
31–								
Nov 3	50 mg							
4	25 mg							
6		278	956	488	660	3960	3640	5744
10–12	ACTH 50 mg							
13	50 mg	230	832	433	648	2 50	1910	38?3
14–19	50 mg							
20	50 mg	193	804	440	548	473	34 7	1827
21–2	50 mg							
23–25	25 mg							
26	12 mg							

lipids and cholesterol decreased concomitantly The marked improvement in serum lipids was accompanied by a complete diuresis with a rise in serum proteins and clinical evidences of well being The result was classified as a partial remission

Case #2 M L M 10 year old female (Table II) who had been well until 1944 showed a similar course in respect to the general trend of lipids although cholesterol and lecithin showed less effect As in the preceding case triglycerides and total lipids declined markedly In this case as in T K diuresis occurred during the experimental period unlike the first case however no significant change was found in the serum proteins Proteinuria continued undiminished

Case #3 D H 13 year old male was taken ill in December 1949 The lipid disturbance as reflected by serum findings was of lesser degree the first control specimen having showed a cholesterol of 435 mgs% Table III shows data collected from the second course of

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Table IV

A G FEMALE 42 YRS

Date	Rx	Cholesterol			Phospholipid (as Lecithin)	Nonphospholipid EFA	Triglycerides	Total Lipid
		Free	Total	Ester FA				
Control		188	756	419	354	676	270	1799
Oct 5–9	ACTH 50 mg							
10	50 mg	252	760	366	482	65	300	1708
11–12	50 mg							
16	50 mg							
17	100 mg	208	752	392	394	602	221	1759
" 18–23	100 mg							
24	100 mg	190	772	419	383	828	429	2003
25–26	" 100 mg							

Table V

R B MALE 48 YRS

Date	Rx	Cholesterol			Phospholipid (as Lecithin)	Nonphospholipid EFA	Triglycerides	Total Lipid
		Free	Total	Ester FA				
Control		145	648	36[illegible]	404	588	237	1650
Oct. 13	ACTH 75 mg							
14–16	100 mg							
17	" 100 mg	113	520	293	331	500	218	1362
18–21	100 mg							
" 22	50 mg							
24		117	546	30[illegible]	381	588	2[illegible]3	1529
Nov 7		106	562	3[illegible]	380	506	186	1457

Table VI

L B, FEMALE 58 YRS

Date	Rx	Cholesterol			Phospholipid (as Lecithin)	Nonphospholipid EFA	Triglycerides	Total Lipid
		Free	Total	Ester FA				
Control		113	408	212	312	671	48[illegible]	1414
Aug 23	ACTH 50 mg							
24–31	100 mg							
Sept 1	50 mg							
5		105	440	242	350	688	469	1501
6–14	100 mg							
15	50 mg	132	450	2[illegible]9	289	737	534	1502
Nov 13		107	412	2[illegible]0	371	792	600	1603

(Table V) showed a decline in triglycerides following therapy a finding which was correlated with a substantial rise in serum proteins This patient had no evidences of edema upon admission and changes in body weight were negligible throughout the course L B (Table VI) demonstrated a brief decline in triglycerides with a final rise

Table II

M L M, FEMALE 10 YRS

Date	Rx	Cholesterol			Phos pholipid (as Lecithin)	No phos pholipid EFA	Triglycerides	Total Lipid
		Free	Total	Ester FA				
Control		235	902	480	462	1225	78?	26 6
Sept. 20	ACTH 25 mg							
21–24	75 mg							
25	75 mg	214	770	401	467	1100	734	2372
26–28	75 mg							
29	50 mg							
Oct 2		197	822	450	395	947	522	2189

Table III

D H MALE 13 YRS

Date	Rx	Cholesterol			Pho pholipid (as Lecithin)	Nonphos pholipid EFA	Triglycerides	Total Lipid
		Free	Total	Ester FA				
Control		96	215	86	222	264	187	710
Oct. 26	ACTH 50 mg							
27–29	100 mg							
30	100 mg	65	302	171	218	228	59 9	751
31–								
Nov 3	100 mg							
4–5	50 mg							
6	50 mg	60	225	119	218	250	123	699
7	50 mg							
13		57	261	147	228	264	123	759

treatment The control values at this time reflected approximately normal values the triglyceride figure of 187 being the only component significantly above normal quotations The first determination of triglycerides following commencement of the ACTH regime showed a dramatic drop reminiscent of T K but this was not maintained on a decreased dose There was more actual fluctuation in the individual lipid fractions than the total lipids which totals fell within 5% of the mean of all Notwithstanding the less conspicuous lipid disturbance and the transient result from ACTH therapy the clinical course of this subject was characterized by complete diuresis with a moderate decrease in proteinuria and a slight increase in serum proteins

Cases #4 5 & 6 A G R B and L B ages 42 48 and 58 respectively have been grouped together because of the common factors of duration of illness and age group A G (Table IV) showed a rise in serum lipids while on therapy This was correlated with failure of diuresis and unaltered proteinuria and hypoproteinemia R B

absorption of intestinal lipids Furthermore Hiller et al[10] concluded that absorption of fat from the small intestine in nephrosis is unaltered

Studies on the adrenal cortex in relation to fat absorption fail to demonstrate an appreciable influence Changes of the magnitude shown in M L M and T K could thus hardly be accounted for by a variation of absorption

Evidence is lacking which would lead us to suspect a defect of carbohydrate metabolism in the nephrotic syndrome capable of accounting for the lipemia Nor do the effects of ACTH on carbohydrate metabolism allow an explanation of the phenomena here described The considerable volume of work on the relation of proteinuria or hypoproteinemia to lipemia has never resulted in a cause and effect relationship In addition the effects which we have shown to occur in these patients and others after ACTH have involved the lipids more promptly and more commonly than the proteins

A decrease in fat oxidation has been ruled out by Hiller et al [10] They have confirmed fat oxidation in post absorption determinations on fat fed nephrotics by showing an R Q depression as in their normals

For these reasons it seems more plausible to consider the possibilities of (1) a decrease in deposition or (2) an increase in mobilization as a source of the lipids which we find pooled in the transport system The second possibility has received considerable support from clinicians who cite the cachexia of the nephrotic patient Such a paucity of subcutaneous fat could however be explained as well by one hypothesis as the other In either mechanism of deprivation of the fat stores the serum triglyceride fraction would be the most significant

Several investigators have reported a notable decrease or complete loss of ability to produce fatty livers in adrenalectomized animals by various experimental methods [13 14 15 16] Goldzieher[17] concludes that adrenal extract causes fixation of blood fat in tissues Kendall has reported increase in carcass fat in rats treated with three pure adrenal steroids [18] Hyperplasia of the cortex as well as Cushing s syndrome is a precursor of obesity of a particular type It would be in accord with these findings that a decrease in triglycerides should follow the activation of the adrenal cortex by ACTH

CONCLUSIONS

1 Partition of lipids in nephrosis during and after administration of ACTH indicates that the triglyceride fraction decreases substantially under activation of the adrenal cortex

in all components The clinical course was correspondingly uneventful These three subjects were chosen not as representative of nephrosis patients in general but of the age group over 40 years

SUMMARY OF RESULTS

Three out of six patients showed a significant decrease in serum cholesterol findings roughly paralleled by the phospholipids The fatty acid esters of cholesterol and the non phospholipid fatty acids concurrently decreased The serum triglycerides on the other hand and with them the total lipids showed a drop in five of six patients a subsequent rise occurred in one subject A G

GENERAL DISCUSSION

It will be noted that our control findings agree with those of other investigators[10,11] Although the majority of our patients have been shown to demonstrate a notable drop in serum cholesterol under treatment with ACTH we have included in this group three subjects in whom a decrease did not appear It is thus evident that triglyceride changes were more closely associated than cholesterol with ACTH induced cortical activity In correlating triglyceride changes with the age of the patient and the severity of the disease we find that the youngest (Table I) reflects the most marked restoration of lipid concentration towards normal On the control determination cholesterol values of 1291 and triglycerides of 4510 were found to indicate the most profound lipid disturbance Phospholipids of 702 and total lipid of 7129 are also extreme elevations In this patient diuresis and clinical improvement were associated with a decrease in cholesterol to 804 but a more striking decrease in triglycerides to 35 mg%

On the other hand L B a woman of 58 years of age who had shown herself to be quite inert to treatment had no decrease in cholesterol and on the other hand demonstrated a definite increase in triglycerides The other patients showed intermediary effects with the serum triglycerides showing the most immediate response

Frazer[1] presents evidence that transportation of lipid in the blood stream is effected mainly as long-chain triglyceride in association with lipoproteins On this basis the triglyceride values can be employed as a quantitative index of fats in transit which values in the nephrotic state indicates a large excess

Five possibilities could be considered to account for triglyceride values in fasting blood greatly exceeding the minimal levels normally present as lipid in transport (1) variation of absorption (2) decrease in oxidation (3) increased synthesis from carbohydrate or protein (4) decline in deposition or (5) increase in mobilization We can disregard increased absorption because of the normal nearly complete

During and for several days following five days of ACTH therapy alone at a dosage of 125 milligrams per day there were striking changes in both the gross appearance (see Fig 1) and chemical analysis of the serum Marked clearing of the serum was apparent by the



Fig 1 14 hour postabsorptive sera from the patient with panhypopituitarism The tube on the right is pretreatment the center and left tubes are 2 and 5 days respectively after completion of 5 days ACTH administration

2 The triglyceride fraction appears to be the most sensitive of the lipid fractions to stimulation of the adrenal cortex

3 Since the triglycerides are primarily concerned with the fat trans port system it is likely that the hyperlipemia of nephrosis stems from a failure to deposit fats or a proclivity towards mobilization It would seem that the action of the stimulated adrenal cortex is ultimately focussed upon the fat depots whether by mobilization or deposition

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DISCUSSION

DR RICHMOND W SMITH JR In recent observations on a 41 year old male with panhypopituitarism of five years duration without treatment the serum total lipids were 1560 milligrams per cent the total and esterified cholesterol were 266 and 187 milligrams per cent respectively and no color reaction took place in the phospholipid phosphorus analysis even though four times the usual volume of serum extract (alcohol ether) was used At this time serum and plasma were highly opalescent The diet throughout was weighed constant and contained 135 grams of fat per day

PART V

VITAMIN METABOLISM

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Factors Which Influence the Concentration of Serum Vitamin A*†

Calvin T Klopp C W Tabor ‡ and A Danish

THE GEORGE WASHINGTON UNIVERSITY MEDICAL SCHOOL WASHINGTON D C

Our initial interest in vitamin A was aroused by an observation made a number of years ago by J C Abels et al [1] namely that 86% of the patients with gastrointestinal cancer had plasma vitamin A levels below normal We confirmed these findings but we noted certain variations in results which could not be explained by the present concept that variations in the concentration of vitamin A in serum reflect primarily dietary influences The following studies were undertaken to define some of the factors which influence the concentration of vitamin A in the blood stream The determinations of vitamin A were done on serum by the method of Sobel and Snow

SHORT TERM CLINICAL STUDIES[3]

As a control the concentration of serum vitamin A in fasting individuals was determined at hourly intervals for six hours

The variations from hour to hour were within the range of plus or minus 50 International Units per 100 cc (International Units/100 ml) (See Fig 1)

* Aided by grants from the National Cancer Institute U S Public Health Service and The American Cancer Society

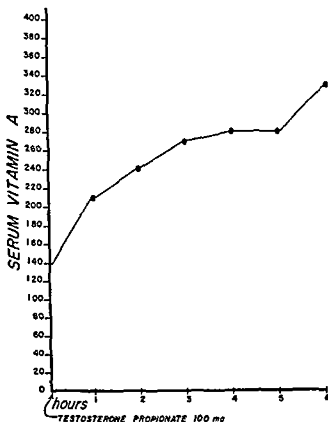
† Testosterone for this study was supplied by Schering Corporation Cortisone Acetate was supplied by Merck and Company aqueous emulsion of vitamin A was supplied by Endo Products Company as Acon vitamin A dissolved in oil was supplied by Lilly and Company as Alphalin ACTH was supplied by The Armour Laboratories

‡ Trainee of American Cancer Society

fourth treatment day, and this persisted for several days after ACTH was discontinued Two days following completion of ACTH administration the total lipids were 1025 total cholesterol 278 and phospholipid phosphorus 13 7 milligrams per cent respectively

This would suggest that the adrenal steroids in this particular instance exerted a definite influence on the utilization and serum distribution of lipids such that the neutral fats the triglycerides were either oxidized or stored and converted in part to phospholipids The clearing of the milky hyperlipemia is most interesting and seems to indicate that the phospholipids were important in determining the physical properties of the circulating lipids Later studies in this patient however suggested that the level of neutral fats was probably equally important in this effect

These remarks although concerning another disease entity seem apropos in view of the above report of Drs Soshea and Farnsworth on the lipid changes in ACTH treated nephrotics



EFFECT OF A SINGLE INTRAMUSCULAR INJECTION OF *TESTOSTERONE PROPIONATE* *(in oil)* ON SERUM VITAMIN *A* CONCENTRATION *(IU/100 ml)*

Fig 2

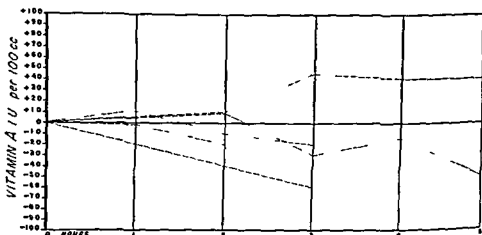
patients and bloods for analysis obtained each hour for four to six hours thereafter The results are shown in Fig 6

In each individual a bi phasic type of response was obtained namely an initial increase in the first hour followed by a rapid de crease in the next with a subsequent return toward the normal levels

A few long term studies were made of the effects of repeated in jections of testosterone It was found that the serum vitamin A con centration could be increased and maintained at that higher level in most cases during the period of administration of the testosterone (Fig 7) As a result of these studies it was postulated that the concen

A single injection of testosterone (50 to 150 mgm) was given to each of 29 individuals and bloods for analysis obtained at hourly intervals for six hours In 26 individuals an increase in concentration of from 50 to 310 International Units/100 ml occurred maximum in six hours A typical response *in one individual is illustrated* in Fig 2

A single subcutaneous injection of 0 3 mg of epinephrine was given to each of 19 patients studied An increase of from 50 to 290 International Units/100 ml was obtained in 16 cases being maximal



VARIATIONS IN SERUM VITAMIN A CONCENTRATIONS IN FASTING NORMAL ADULT INDIVIDUALS

FIG 1

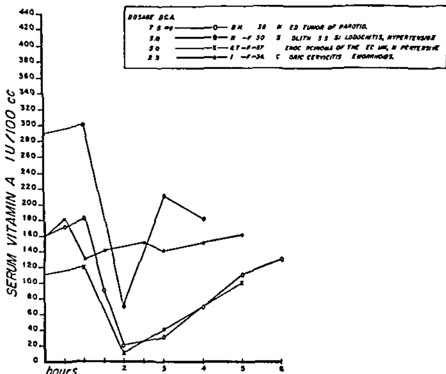
in two hours A typical response in one individual is illustrated in Fig 3

A single injection of desoxycorticosterone (2 5 to 7 5 mgm) was given to each of the four individuals There occurred within two hours a decrease of from 60 to 225 International Units/100 ml in the concentration of serum vitamin A followed by a return toward normal values (Fig 4)

A single intramuscular injection of Cortisone Acetate (25 to 100 mgm) was given to each of four individuals An increase of from 60 to 240 International Units/100 ml in serum vitamin A concentration occurred in three of the four cases (Fig 5) The fourth individual was a patient with terminal carcinoma of the cervix who was found to have evidence of nitrogen retention on the day the test was conducted

A single injection (25 mgm) of ACTH was given to each of four

summarized in Fig 8 For this experiment a total of 120 animals of the Sprague Dawley strain divided into two groups of 60 each were used Each animal in one group was pair fed with a corresponding animal in the second group and given equivalent amounts of drinking water and 0 1% saline solution Each animal in both groups re

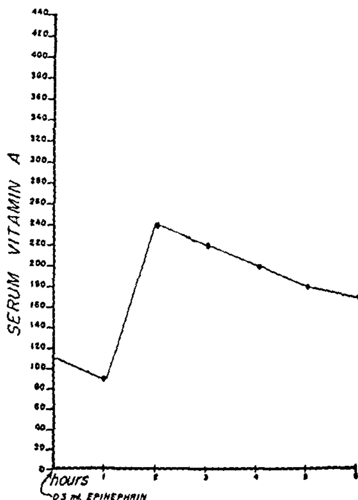


EFFECT OF A SINGLE INTRAMUSCULAR INJECTION OF *DESOXYCORTICOSTERONE ACETATE ON* SERUM VITAMIN *A* CONCENTRATIONS IN FASTING INDIVIDUALS

Fig 4

ceived 500 units of an aqueous emulsion of vitamin A subcutaneously daily

The sixty animals in Group I were subjected to a sham adrenalectomy and the sixty in Group II to a bilateral adrenalectomy Six animals in each group were sacrificed daily Bloods for analysis were obtained by cardiac puncture at the time of sacrifice The sham operated animals maintained a normal or perhaps slightly elevated vitamin A concentration while in the adrenalectomized animals the



EFFECT OF A SINGLE SUBCUTANEOUS INJECTION OF 0 3 mg OF EPINEPHRINE (1 1000) ON SERUM VITAMIN A CONCENTRATION (I U/100 ml)

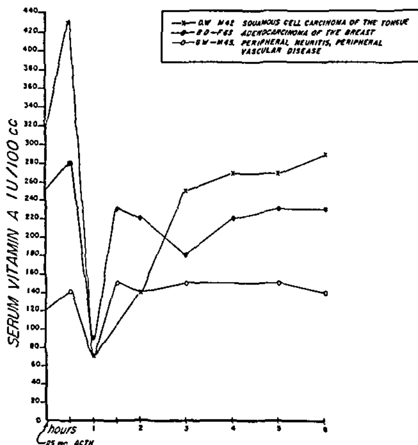
Fig 3

tration of vitamin A in blood reflected in many instances hormonal factors rather than diet.

ANIMAL STUDIES[4]

In an attempt to define some of the hormonal factors involved further studies were undertaken in the rat and the dog

The important results obtained from the rat experiments are



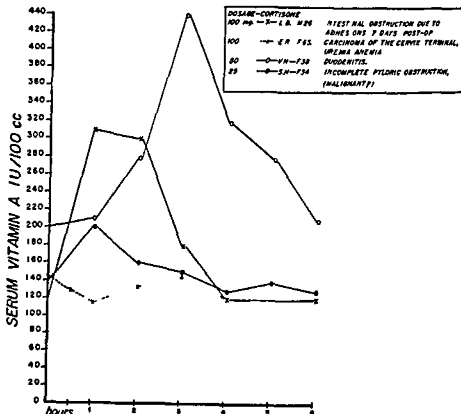
EFFECT OF A SINGLE INTRAMUSCULAR INJECTION OF ACTH ON SERUM VITAMIN A CONCENTRATIONS IN FASTING INDIVIDUALS

FIG 6

In the animals whose right adrenal had been removed the serum values did not return to the preoperative level but were maintained at a lower level for the interval before the second operation During this interval the animals received no therapy except for normal saline which was substituted for drinking water

At the end of two weeks the remaining adrenal was removed and the vitamin A concentration decreased to negligible values The animals survived about 24 hours and during that time no vitamin A could be found in the blood (Fig 9)

ACTH was then administered in 5 mgm doses to two normal dogs



EFFECT OF A SINGLE INTRAMUSCULAR INJECTION OF CORTISONE ACETATE ON CONCENTRATION OF SERUM VITAMIN A IN FASTING INDIVIDUALS

FIG 5

serum vitamin concentration decreased within ten days to insignificant levels

Therefore the presence of a normal adrenal gland was necessary to maintain normal concentration of vitamin A in the blood of the rat

Daily determinations of the concentration of vitamin A in the serum of the dog were made in animals subjected to a bilateral adrenalectomy in stages The right adrenal was removed first and the left adrenal approximately two weeks later As controls animals were used which had been subjected to a thoracotomy In both groups of animals there occurred at the time of operation a sharp drop in serum vitamin A concentration which returned to the normal values in the control animals within a few days

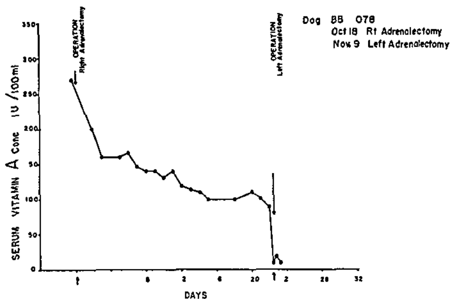


FIG 9

SERUM VITAMIN A Conc IU/100ml
350
300
250
200
150
100
50
0
0 4 8 12 16 20 24 28 32
DAYS
ACTH mgm IM/day
20
0

FIG 10

EFFECT OF INJECTIONS OF TESTOSTERONE PROPIONATE ON SERUM VITAMIN A CONCENTRATION IN PATIENT WITH COLON CANCER

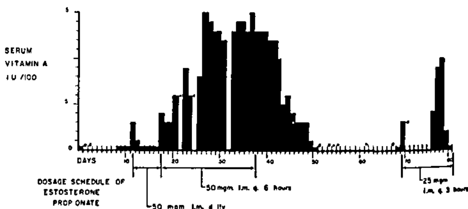


FIG 7

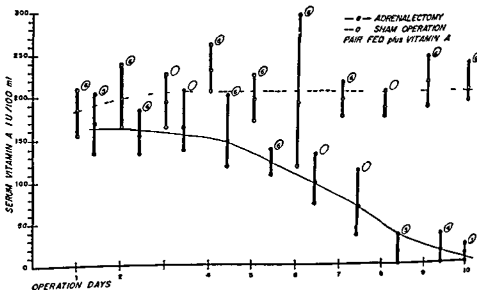


FIG 8

every 12 hours for ten days and bloods for analysis obtained daily

The animals were fed a relatively constant diet Following the administration of ACTH the serum vitamin A concentration decreased to about half the original value and was maintained at that level during the period of therapy as demonstrated in Fig 10

To evaluate these observations 18 adrenalectomized rats divided into three groups of six were studied In the first group no additional therapy was given One animal in each of the other two groups (B and C) was pair fed with a corresponding animal in group A In addition each animal in group B received 500 units of vitamin A daily and each animal in group C 0 5 cc of saline The adrenalectomized animals receiving vitamin A lived 9 days longer and were in much better condition throughout the experiment than those which had

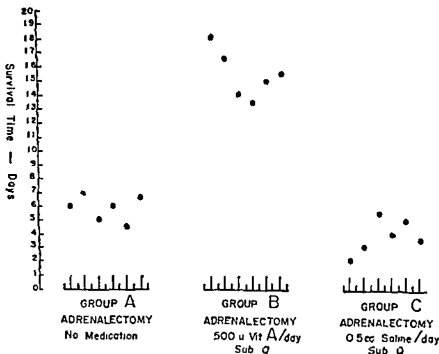


Fig 12

not received it (Fig 12) The probability that the increased survival time was due to chance was less than 1 0% All animals were caged in the same room but in individual cages and were tended by the same technician

SUMMARY

As a result of these studies we feel that while we do not know the mechanisms involved at least one fat soluble vitamin must be considered in the evaluation of endocrine studies especially those involving the pituitary adrenal system

Realizing that the rat and the dog were unable to maintain vitamin A in the blood when the adrenals had been removed it was important to determine if the concentration of vitamin A in liver was affected Three groups each consisting of six adrenalectomized rats were studied for 6 days Two of these groups received subcutaneously 500 units of vitamin A daily as an aqueous emulsion in one and dissolved in oil in the other The third group received no therapy Each animal in each of the groups receiving vitamin A was pair fed with a corresponding animal in the group which had received no vitamin A

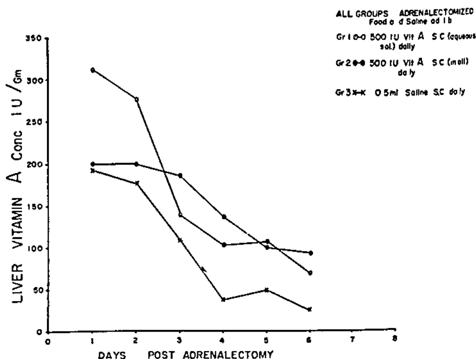


Fig 11

min A One of each group was sacrificed daily for this experiment and liver tissue obtained for analysis Within six days the vitamin A content of the liver expressed in units per gram had decreased to approximately 20% of the normal value (Fig 11)

In the course of these experiments we noted that in most instances the adrenalectomized rats which had been given vitamin A lived several days longer under the conditions in our laboratory than did those adrenalectomized rats which had not received vitamin A In addition the adrenalectomized rats receiving vitamin A were more alert had glossier coats better wound healing and less evidence of wound infection than those not receiving vitamin A

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The Effect of Adrenocorticotrophic Hormone and Cortisone Acetate on the Metabolism of Ascorbic Acid*†

J C Beck J S L Browne and K R Mackenzie

ROYAL VICTORIA HOSPITAL AND MCGILL UNIVERSITY MONTREAL

Andreae and Browne[1] of this Clinic reported that in traumatized previously healthy individuals there was a fall in the blood and urinary ascorbic acid accompanied by a marked rise in the urinary corticoids They also noted that large quantities of ascorbic acid were required to increase the urinary excretion of this substance in these subjects

The concentration of ascorbic acid in the adrenal has been shown to be higher than in any other tissue[3] Sayers Long and their collaborators[4,5] have shown that following ACTH in the rat and the guinea pig there is a depletion of the adrenal ascorbic acid to two thirds its control level in 20 minutes and that a gradual restoration of the ascorbic acid occurs over the following 24 hours They also note that the rate at which restoration occurs is less in the guinea pig and feel that this is associated with its incapability of synthesizing ascorbic acid A variety of stresses causes a similar reduction in the adrenal ascorbic acid content[6,7]

The impression that one gets from these varied observations is that ascorbic acid is important to the adrenal cortex Thus on the basis of these observations and in conjunction with investigation of the general metabolic effects of ACTH and cortisone it was felt desirable to observe the changes in ascorbic acid metabolism associated with the administration of these hormones

Daily urinary and regular blood ascorbic acid levels were observed in patients with a variety of chronic disease states (Table I) to which ACTH and cortisone were administered using the technique of Roe and Kuether[8] based on the estimation of the 2 4 dinitrophenylhydrazine derivative of dehydroascorbic acid

* This work was supported by a grant from the National Research Council (Canada)
† This work was supported by generous allotments of ACTH from The Armour Laboratories The authors are also indebted to Hoffman-La Roche Limited and to Dr R L Wolfe for the generous supply of ascorbic acid used in this study

see little Addison s disease We have had one Addison s case in this series on whom we did run an adrenalin test At the time of our last tabulation that case represented the only instance in which we did not get any increase in vitamin A concentration in two hours We have had cases in which the amount of the increase was not significant

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Table I

TYPES OF CHRONIC DISEASE

	Processes Investigated	Number of Cases
1	Rheumatoid Arthritis	4
2	Rheumatoid Arthritis and Psoriasis	1
3	Rheumatoid Arthritis and Bronchial Asthma	1
4	Bronchial Asthma	8
5	Rheumatoid Arthritis and Multiple Myeloma	1
6	Chronic Berylliosis	2
7	Silicosis	1
8	Hodgkins lymphoma and Amyloidosis	1
9	Periarteritis Nodosa	1
10	Malignant Exophthalmos	1
11	Ulcerative Colitis	1
12	Lupus Erythematosis	2
13	Pernicious Anemia	1
14	Sympathetic Ophthalmia	1
15	Atopic Dermatitis Keratoconus	1
16	Addison s Disease	1

Twenty nine patients were placed on one of three levels of ascorbic acid intake Nineteen patients received an intake of approximately 250 mg per 24 hr two received approximately 1000 mg /24 hr and in 8 no supplement was given and the ascorbic acid content of the diet was kept as low as was feasible taking into consideration the other metabolic studies which were being carried out ACTH dosage varied between 20 and 200 mg /day in four equally divided injections Cortisone acetate as a microcrystalline suspension in saline was given intramuscularly in 50 to 500 mg doses per 24 hours

The findings are best discussed by grouping the patients according to their ascorbic acid intake and according to whether they received a course of ACTH or cortisone

1 Fifteen patients on an ascorbic acid intake of 250 mg /24 hr received ACTH *Twelve of these showed a sharp and in 3 cases* maintained increase in the urinary excretion of ascorbic acid during the first 24 to 48 hours of ACTH administration (Fig 1 2 3) two showed a questionable increase and in one no effect upon the urinary excretion of ascorbic acid was noted On reduction of ACTH dosage below approximately 50 mg /24 hr or upon cessation of ACTH administration 14 patients showed a marked diminution in the urinary ascorbic acid output lasting 1 to 3 days In one patient during 4 days

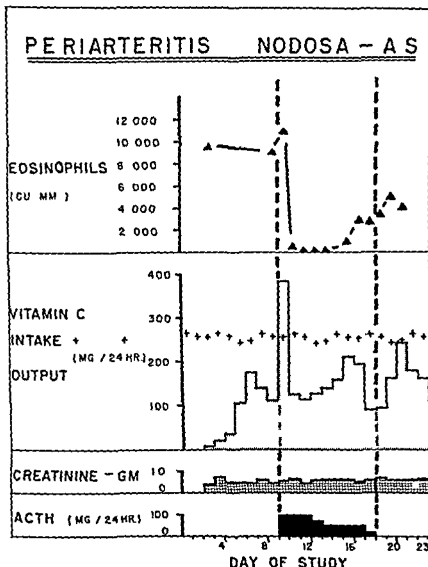


FIG 1 Increased urinary ascorbic acid excretion after ACTH with a diminution in excretion upon cessation of ACTH administration

of ACTH administration there was an ascorbic acid excretion of 800 mg above the average control level Eleven of these subjects were in negative ascorbic acid balance during the initial period of ACTH administration

2 Four patients on an ascorbic acid intake of 250 mg /24 hr received cortisone acetate In 2 of these there was a questionable increase in the urinary ascorbic acid excretion and in 2 no effect on the

MALIGNANT EXOPHTHALMOS — C C

Fig 2 A maintained increase in ascorbic acid excretion during ACTH administration with diminished output upon cessation of ACTH

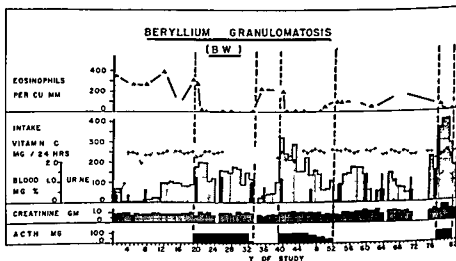


Fig 3 A suggestion that this effect may be potentiated by repeated courses of ACTH administration

urinary output could be demonstrated No alteration in the ascorbic acid excretion occurred on cessation of the cortisone administration in any of these cases One of these patients with well established Addison s Disease was given 100 mg ACTH/24 hr for 2 days with no

effect on the urinary excretion of ascorbic acid Although no conclusions can be drawn from one case it suggests that ACTH has no direct effect on ascorbic acid excretion not mediated through the adrenal

3 Seven patients on an unsupplemented ascorbic acid intake averaging between 15 and 90 mg /24 hr in respective studies received ACTH (Fig 4) Four of these showed an increased urinary output of ascorbic acid upon ACTH injection one showed a questionable in

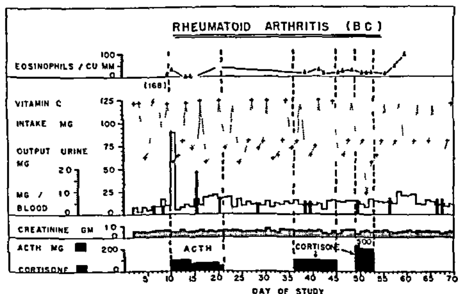


Fig 4 A typical response after ACTH administration in a patient on an unsupplemented ascorbic acid intake with a failure of an increase in urinary ascorbic acid excretion after cortisone

crease and in 2 no response to the administration of ACTH was discernible Three of the 4 subjects who showed increased ascorbic acid output showed a subsequent decrease on reduction or cessation of ACTH administration as did the one case with a questionably increased output Those showing no effect on ACTH administration showed no effect on withdrawal of the hormone

4 One patient (Fig 5) on no ascorbic acid supplement and who previously had shown a characteristic response to ACTH administration received 2 courses of cortisone injections and showed a step wise increase in urinary ascorbic acid on both occasions No alteration in excretion occurred on cessation of cortisone administration Three patients on this intake level received cortisone subsequent to a course

Fig 2 A maintained increase in ascorbic acid excretion during ACTH administration with diminished output upon cessation of ACTH

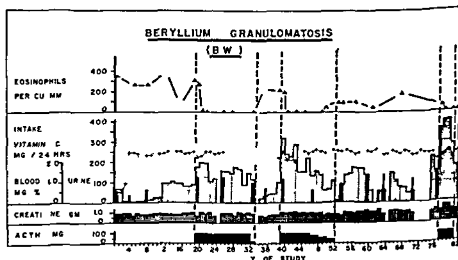


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A 9 month old infant* with clinical and X ray evidence of advanced scurvy was placed on a Vitamin C free diet and received 30 mg ACTH for 24 hours (Fig 6) Urinary ascorbic acid excretion initially below 0 5 mg /24 hr increased to 2 3 mg /24 hr coincident

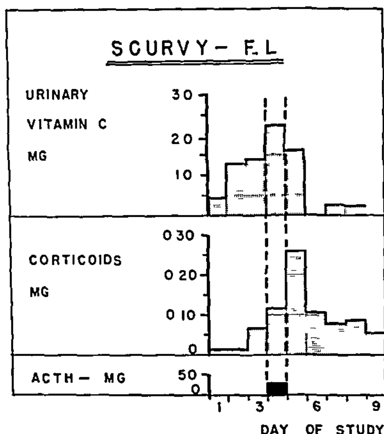


FIG 6 Urinary ascorbic acid and urinary corticoids in an infant with scurvy receiving ACTH

with a rise in the chemically determined corticoids The blood ascorbic acid remained zero during the period of study Following this short course of ACTH injections there occurred definite clinical improvement corroborated by calcification in the femoral sub periosteal hematomata (Fig 7 8)

This data further substantiates the impression that ascorbic acid

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*This patient was studied in collaboration with Dr G Morris and through the courtesy of Dr A Goldbloom of the Childrens Memorial Hospital

of ACTH and in none was any effect on ascorbic acid excretion discernible (Fig 4)

5 One patient on an ascorbic acid intake of 1000 mg /24 hr receiving ACTH showed an increased excretion of ascorbic acid in response to the ACTH but no fall was noted upon cessation of the injections

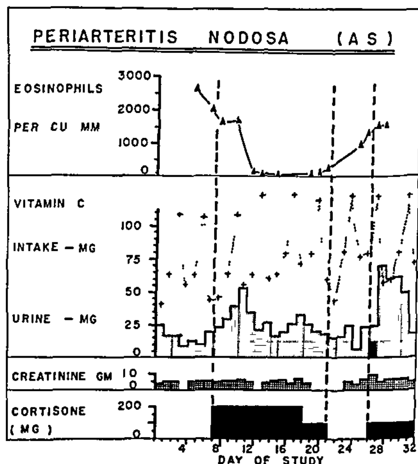


Fig 5 A demonstration of a urinary ascorbic acid response after cortisone

6 One patient on an ascorbic acid intake of 1000 mg /24 hr and receiving cortisone showed no alteration in ascorbic acid excretion either upon initiation or cessation of the injections

Regular blood ascorbic acid levels were determined and the impression conveyed suggests that these levels increase when the urinary ascorbic acid excretion is increased and that they remain low or constant when the urinary ascorbic acid excretion is low or unchanged

cretion occurred In only a few patients was this increase maintained during the remaining period of ACTH injection In general those patients showing a peak in ascorbic acid excretion display a definite diminution of variable magnitude in the urinary ascorbic acid excretion upon reduction or cessation of ACTH dosage The dosage level of ACTH at which this occurs appears to be approximately 50 mg per 24 hr In the low ascorbic acid intake group there was a suggestion that if the ACTH dosage was high enough the peak in the urinary ascorbic acid excretion would occur

Nine patients received cortisone of these 1 showed a definite increase in the excretion of ascorbic acid 2 showed questionable increases and in 6 no effect on the urinary ascorbic acid output was noted In the one response the increased excretion was of a step-like nature suggesting that the effective cortisone level to produce this phenomenon was reached slowly This is in agreement with Browne s opinion that the micro-crystals of cortisone acetate are only slowly absorbed and that the actual dose over a given period of time is far removed from the injected dose Thus a series of injections has a potentiating effect so that a physiologically effective dosage is reached

The failure of cortisone to produce this peak effect in a larger number of cases may be merely a question of inadequate dosage due to two factors the first of which has just been discussed The second factor is associated with evidence now beginning to accumulate that ACTH is four to five times as effective in releasing endogenous cortisone or cortisone like substances than an equal amount of injected cortisone acetate This difficulty could be answered in part by the administration of soluble cortisone

That this response is mediated through the adrenal and is not a direct effect of ACTH is suggested by the failure of ACTH to cause an alteration in ascorbic acid excretion when administered to a patient with Addison s disease although no conclusions can be drawn from one case

The source of the increased urinary ascorbic acid following ACTH in the scorbutic infant and the reason for the clinical and X ray evidence of improvement may be associated with the release of ascorbic acid from the adrenal cortex in response to ACTH stimulation Harris[9] has shown that in scurvy the adrenal is not completely depleted of ascorbic acid

The cause of this peak in urinary ascorbic acid excretion can only be hypothecated at present but several possibilities exist

(1) That it is due to an increased renal clearance of ascorbic acid associated with a lowering of the threshold after ACTH Ascorbic or hexuronic acid is a 6 c atomed compound closely allied to glucose McAlpine Hoffman et al[10] demonstrated that part of the glycosuria

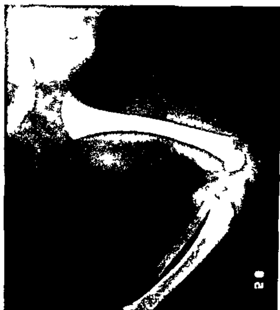


FIG 7 X ray film of scorbutic infant before ACTH administration



FIG 8 X ray film 14 days after administration of 30 mg ACTH and 6 days after a single injection of 15 mg ACTH

is important in adrenal cortical physiology the role it plays can still only be hypothecated

The results of this investigation are summarized in Table II but may be briefly mentioned Of the 23 patients receiving ACTH 17 showed a sharp increase in the urinary excretion of ascorbic acid during the first 24 to 48 hours of ACTH administration 3 showed a questionable increase and in 3 no alteration in urinary ascorbic acid ex

Table II

Ascorbic Acid	*Patients receiving ACTH (23)*			*Patients receiving cortisone (9)*		
	*Response**	*Questionable Response*	*No Response*	*Response**	*Questionable Response*	*No Response*
Intake with no supplement	14	1	2	1	–	3
250 mg Intake	12	2	1	–	2	2
1000 mg Intake	1	–	–	–	–	1
	17	3	3	1	2	6

* Response indicates an increase in urinary ascorbic acid excretion upon administration of ACTH or cortisone.

The failure of a larger number of responses with cortisone tend to rule out a peripheral cellular effect as a source of ascorbic acid rather than an adrenal cortical although the apparently definite response obtained in one case tends to throw some doubt on this premise

An attempt to understand the decrease in urinary ascorbic acid upon reduction in or cessation of ACTH administration can only be made by comparing it to the blood sugar overswing which is seen in continuous carbohydrate administration It seems that one metabolic pathway once established has precedence over another

Further investigations along lines of obtaining adrenal blood after the administration of these hormones would certainly promote the understanding of this problem

This discussion is largely speculation and perhaps no one hypothesis offers the answer to the problem but a combination of factors may be at work No final conclusions can be reached on the basis of the available data

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occurring during ACTH administration was due to a lowering of the renal threshold This observation tends to support the hypothesis but if this is true it seems difficult to understand that the increased ascorbic acid excretion does not continue for the duration of ACTH administration There is also a rise in the blood ascorbic acid during the period of ACTH administration which would not occur were this increased excretion due to a lowered renal threshold Finally one would expect a higher number of urinary ascorbic acid peaks than is demonstrated with cortisone because the dosage given is adequate to result in glycosuria Renal clearance studies would be of great value in solving this phase of the problem

(2) That the peak be due to rapid and continued release of ascorbic acid by the adrenal The work of Sayers and co workers[11] is excellent evidence in favour of this They observed a fall in ascorbic acid and cholesterol content of the adrenals of rats after ACTH injection The fall was maximal at one hour remained there for two to three hours returned to its original level after nine hours and finally after 24 hours reached a greater level than in controls This suggests that the adrenal cholesterol and ascorbic acid is essential to the synthesis of the cortical hormone Lowenstein[12] reported isolation of a biologically active adrenal steroid in which the side chain was *conjugated with ascorbic acid conferring water solubility to the compound* Giroud[13] has suggested that the synthesis of the cortical hormone or hormones is dependent upon the presence of ascorbic acid in the adrenals it playing an important role in the oxidation reduction process in this organ It is possible that the ascorbic acid facilitates certain types of chemical reactions within the cells of the adrenal cortex or possibly within all cells upon which the adrenal hormone exerts its action

It has been suggested that the large quantities of ascorbic acid which appeared in this investigation could not possibly have the adrenal as its only source and that other tissues also release ascorbic acid in response to ACTH administration A similar statement might be made with regard to the urinary corticoids after ACTH administration but it is postulated that these are synthesized in the adrenal cortex Could it not also be possible either for ascorbic acid to be withdrawn from other tissues and transported to the adrenal for a more vital function or for synthesis of it to occur in the adrenal? Only further investigation along these lines can elucidate this problem

(3) That the source of the increased urinary ascorbic acid be a general release of ascorbic acid from some other organ or tissue or that a combination of these factors play a role in production of this peak

guinea pig there is ample evidence of the secretion of adrenal steroids after the administration of ACTH

Then there was the work of Voight who showed that in the ad renal vein there is no increase in ascorbic acid content at the time there was an increase in the steroid content

In our own work with the chick we have shown that both stress and ACTH would cause no decline in adrenal ascorbic acid while there was a decline in the adrenal cholesterol

EFFECT OF CORTISONE AND ACTH ON BODY WEIGHT AND SURVIVAL OF SCORBUTIC GUINEA PIGS

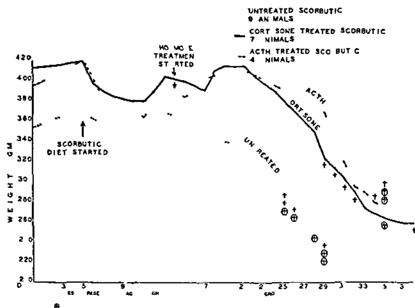


FIG 9

DR M C ROSENTHAL (New England Medical Center and Tufts College Medical School Boston) Our interest in the effect of ACTH on as corbic acid metabolism began after the development of a hemor rhagic syndrome in a patient being treated with this drug for lym phosarcomatosis

Following 115 days of continuous ACTH therapy this patient displayed numerous petechiae and ecchymoses not associated with trauma The platelet count at that time was 400 000 and investigation of the various coagulation factors and their possible antagonists

10 McIntosh H W McAlpine H T Singer B and Hoffman M M Administration of ACTH to Normal Individuals and Patients with Intra or Extrasellar Pituitary Tumours Proceedings of the First Clinical ACTH Conference P 15 Blakiston Co Philadelphia 1950
11 Sayers G et al Effect of Adrenotrophic Hormone on Ascorbic Acid and Cholesterol Content of the Adrenal Proc Soc Exp Biol and Med 55 238 1949
12 Lowenstein B E and Zwemer R D Isolation of a new Active Steroid from the Adrenal Cortex Endocrinology 39 63 1946
13 Giroud A et al Dependance de l hormone corticale vis a vis de l acide ascorbique Compt rend Soc de Biol 134 100 1940

DISCUSSION

DR CHARLES RAGAN Following the work of Schofenberg et al Proc. Soc Biol & Med 74 358 in 1950 in which they reported that cortisone ameliorated the symptoms of scurvy in the guinea pig we repeated their work with both cortisone and ACTH and found that ACTH as well ameliorated the symptoms in the guinea pig in the sense that the lesions of scurvy were less marked and the animals survived for a longer period of time In Figure 9 it is shown that the survival time of the ACTH and cortisone treated animals was considerably longer than that seen in the untreated controls It was of some interest to note that the adrenals of all the controls contained no detectable amount of ascorbic acid This applied as well to the adrenals of the ACTH treated animals In two of the cortisone treated animals there was a minimal amount of ascorbic acid

Fig 10 shows the ratio of adrenal weight to body weight in the normal the untreated scorbutic in which there is a large increase in the adrenal size in the cortisone treated scorbutic in which the adrenal size approaches the normal and the ACTH treated where the adrenal is maximal

The implications we have drawn from this are two fold First in order to have a release of corticoids from the adrenals following ACTH stimulation no or very small amounts of ascorbic acid may be necessary Second during the hyperadrenal state less ascorbic acid is necessary

DR JOSEPH W JAILER To get back to the problem of whether ascorbic acid is necessary for the synthesis or release of the adrenal steroids there are several pieces of evidence to show that it is not

The first is the work of Long who showed that in the scorbutic

ascorbic acid metabolism encountered by other workers It is our feeling that in patients with a potential hemorrhagic tendency due to a deficiency of one or other factors in the hemostatic mechanism treatment with ACTH in certain instances may precipitate an actual hemorrhagic diathesis We attribute this to ascorbic acid depletion with its attendant effect upon capillary integrity

DR ROBERT KARK During the past war Dr Robert Johnson and myself studying soldiers under stress such as a three month march through the Arctic found a marked retention of ascorbic acid at low levels of intake

In attempting to repeat this work recently on healthy individuals medical students and patients with hernias we have found the following to hold

If we saturated our patients with large doses of ascorbic acid before giving them the ACTH we had results which were exactly comparable to those shown by Dr J S L Browne s group that is a large excretion of ascorbic acid

If these men were not saturated sometimes we would get a large excretion but very often a low excretion

We feel that when the subjects are saturated with ascorbic acid ACTH produces an ascorbic acid diuresis on the basis of a lowering of the renal threshold

DR S HOWARD ARMSTRONG JR In view of the fact that a good many of the diseases e g lupus erythematosus and acute rheumatic fever which are affected clinically by ACTH have been reported to show low blood ascorbic acid levels in the last year Dr R Gordon Brown of our clinic has run a group of loading tests on a small number of patients with collagen disease Some of these patients were on good dietary ascorbic intake and some on a low though not completely deficient dietary intake The scatter of the results of the loading tests (using 250 mgs) in these patients did not deviate very much from the scatter obtained from normals nor was there any consistently lower initial blood level observed No more significant results were obtained by using a very high load namely 2 grams intravenously

We thus came to the conclusion that at least from the clinical point of view the problem of interaction of ascorbic and ACTH could not be evaluated without pre and post treatment tissue analyses If our biochemical team is not decimated by the current war we plan to get at this problem this year

conducted by Dr Mario Stefanini revealed a slightly decreased prothrombin activity as well as a slightly increased fibrinolytic activity

These findings however did not adequately explain the severe hemorrhagic manifestations displayed by the patient It was suggested that a possible scorbutic like state existed and the ascorbic acid levels in the urine and plasma were then taken The average plasma ascorbic acid level for four consecutive days was 0 24 mgs per cent The normal for the method being used was 0 7 to 1 0 mg per cent

Urinary excretion of ascorbic acid for this patient over a 24 hour

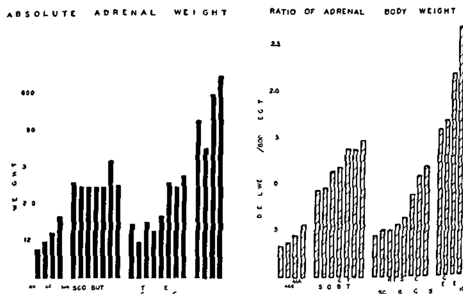


FIG 10

period was 0 99 mgs the normal being 20 to 30 mgs A vitamin C tolerance test showed marked unsaturation of the tissue Administration of vitamin C to this patient completely eliminated the hemorrhagic diathesis

Soon afterward another patient with rheumatoid arthritis presented himself with a similar picture of low ascorbic acid levels in plasma and urine as well as a hemorrhagic diathesis Investigation of other patients treated with ACTH over an extended period revealed that ascorbic acid depletion is not always a constant finding and that furthermore when ascorbic acid depletion does exist hemorrhagic phenomena *are not* a common accompaniment

This possibly explains discrepancies in the effect of ACTH on

PART VI

ADRENAL GLAND AND HEMOPOIETIC SYSTEM

36

The Use of ACTH in Acute Viral Hepatitis*

James W Colbert, Jr † James F Holland Capt (MC) U S A
Ivan Heissler and Marjorie Knowlton
U S ARMY MEDICAL CORPS

The reports of the effectiveness of ACTH against many diseases resistant to all previous therapeutic measures[1 2] suggested a trial of ACTH in the management of acute viral hepatitis The following is a report of the investigation of the use of ACTH in treatment of viral hepatitis at the Hepatitis Center 98th General Hospital U S Zone of Occupation Germany

At this center over 4000 military patients with viral hepatitis have been treated for this disease during the past 3 years 1947–50 The accumulated experience of these three years has been rather carefully reviewed and several reports have been published[3]

MATERIALS AND METHODS

Subjects

The patients studied in this investigation were 5 male enlisted personnel of the United States Army of Occupation in Germany who were admitted to the hepatitis wards of the 98th General Hospital within the first eight days of the onset of viral hepatitis It is possible that some of these cases were examples of homologous serum

From the 98th General Hospital (Hepatitis Research Team) European Command U S Army of Occupation in Germany Representing work done under the auspices of the Commission on Virus and Rickettsial Diseases Armed Forces Epidemiological Board Office of The Surgeon General U.S Army Washington D C and under the auspices of the Section of Preventive Medicine Yale University School of Medicine

† Now with the Department of Internal Medicine Yale University School of Medicine

though jaundice receded during treatment no definite change in liver size or tenderness was noted

There was a clear effect upon the level of the serum bilirubin In all cases serum bilirubin levels showed a prompt fall and upon cessation of ACTH therapy 2 patients exhibited a significant rise in serum bilirubin concentration (Fig 1 & 2) The cephalin flocculation and thymol turbidity tests did not follow as clear a trend However there was a suggestion of an effect of ACTH in returning these tests to normal Gamma globulin levels[5] appeared to be depressed

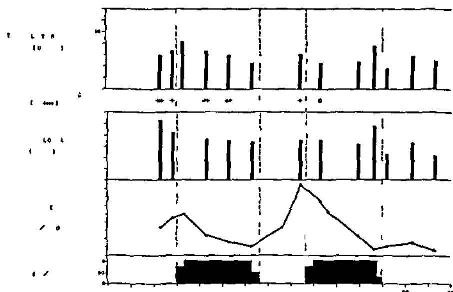


FIG 1 Effect of ACTH on the course of viral hepatitis in patient

slightly by the action of ACTH but again this effect was not conclusively demonstrated Bromsulfalein retention (5 mgm/km of body weight) which was determined after the one minute serum bilirubin had fallen to 0 4 mgm% was abnormal at first examination in all cases

Two patients (R T and A E) experienced clinical relapse upon withdrawing ACTH (Fig 1 & 2) In both instances the relapse was more severe than the initial episode Anorexia nausea and abdominal cramps were accompanied by rise in total and one minute serum bilirubin return to abnormal reaction of the cephalin flocculation and thymol turbidity tests One of these patients (R T) was given ACTH again and within 6 hours nausea and abdominal discomfort vanished his appetite became better and he generally improved By

hepatitis but in view of the fact that there are no means at present for differentiating infectious hepatitis from serum hepatitis clinically the cases have all been classified as viral hepatitis The diagnosis was made on the basis of characteristic symptoms and signs accompanied by consistent deflection of appropriate tests of hepatic function The five patients studied were jaundiced at the time of admission and the subsequent course of disease was mild or moderately severe All patients were under 25 years of age

Management of Patients

The patients were examined daily and observations concerning pertinent symptoms and signs of disease were made Laboratory tests of liver function were determined on admission and twice a week throughout the period of hospitalization Before ACTH was started a regimen of low salt diet daily charting of weight blood pressure intake and output and eosinophil count[4] was instituted Complete blood count urinalysis and glucose tolerance tests were done before treatment and weekly thereafter The urine was examined daily for glucose

ACTH was administered intramuscularly 25 mgms every 6 hours and was discontinued abruptly The total dose per patient varied between 825 mgm and 1925 mgm of ACTH

Except for the administration of ACTH the patients followed a standard management routine The diet contained 4200 calories and consisted of 190 grams of protein 607 grams of carbohydrate and 114 grams of fat supplemented with vitamins and brewer s yeast but restricted in salt intake All patients were kept in bed with the privilege of going to the bathroom until all symptoms and signs of hepatitis had disappeared and the retention of bromsulfalein in the blood measured 6 0% or less They were then allowed to be ambulatory for one week and if there was no evidence of relapse either clinically or as determined by tests of hepatic function they were discharged from the hospital either to duty or to a convalescent center

RESULTS

Appropriate response of the eosinophils to ACTH administration was seen in each instance

The most striking result was the prompt return of appetite and energy Anorexia diminished rapidly and was replaced by a voracious appetite The patients felt so well that it became difficult to maintain bed rest Pruritus when present disappeared during therapy Al

to require narcotic medication This patient experienced remission within 48 hours after ACTH administration was reinstituted

Because the number of patients reported is small detailed case records are given below

Case 1

P A a 19 year old white infantry soldier was admitted to the Hepatitis Center on 11 May 1950 because he was noted to have scleral icterus He was well until 4 days before admission when fatigue dark urine post prandial nausea and anorexia appeared For three days prior to entry intermittent frontal head ache and dull epigastric pain were noted and on the day of admission jaundice was noticed

He drank 5–6 bottles of beer weekly he had never entered the tropics nor had he been exposed to hepatotoxic chemicals There had been no prior jaundice and he had had no needle punctures in the past 6 months The rest of the per sonal past and family histories were essentially negative

On admission the temperature was 98 2 F pulse 64 respiration 20 and BP 138/84 Icterus of the skin and sclerae was present The heart and lungs were normal The abdomen was flat and muscular the liver extended only to the costal margin and was slightly tender the spleen was not palpable The remain der of the physical examination was within normal limits

Hgb was 15 3 gm WBC 10750 with N 59 (0–10–49) L 29 M 11 E 1 Except for bilirubinuria the admission urinalysis was within normal limits A chest X ray was normal A cardiolipin test was negative Liver function tests revealed a total serum bilirubin of 6 3 mgm/100 cc +++ cephalin flocculation 14 thymol turbidity units and 16 5 gamma globulin turbidity units

On the 7th day of disease the patient was started on ACTH 25 mg 4 x daily by intramuscular injection Serum bilirubinuria declined to a concentration of 1 26 mgm % in 12 days and appetite became enormous The appearance of a rounded full plethoric moon facies on the 11th day of ACTH administration was striking After 1675 mg of ACTH administered in 18 days the drug was discontinued because of the appearance of sacral edema significant diastolic hypertension and an acneform eruption over the upper anterior chest and back beginning on the 18th day of treatment From the 13th to 18th day of treatment ascites was noted

Starting approximately 16 hours after cessation of ACTH the patient ex perienced bilateral aching pains in both knees which interfered with sleep but which spontaneously disappeared in 48 hours No objective signs of bone or joint disease were appreciated on examination The sacral edema cleared rapidly as did the rounded facies but acne of the chest persisted for 3 weeks after the ces sation of medication Diastolic hypertension which appeared during ACTH per sisted for one month after stopping the drug On about the 15th day of ACTH therapy and again 10 days after cessation he had brief epistaxes from the left nares

Serial electrocardiograms before during and after the drug failed to show significant changes

Case 2

J L a 23 year old Army clerk was admitted to the Hepatitis Center on 17 May 1950 because of icteric sclerae Three days before admission he observed

the 72nd hour he was energetic and cheerful and his total serum bilirubin had fallen markedly Upon discontinuing ACTH for the second time this patient again exhibited a rise in serum bilirubin but of minor degree (Fig 1) The other patient was treated routinely without ACTH and remitted spontaneously but without dramatic suddenness (Fig 2)

Untoward effects were noted in all 5 patients Ascites sacral edema moon face and acne developed in 4 patients Ascites moon face and sacral edema disappeared with the diuresis that accompanied hormone withdrawal

Rise in systolic blood pressure to a level of over 140 mmHg de

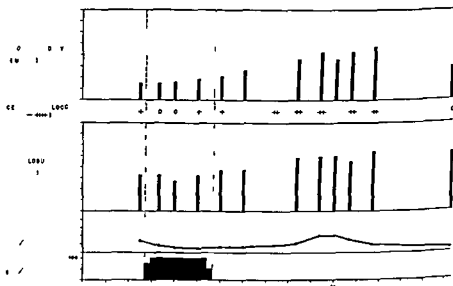


Fig 2 Effect of ACTH on the course of viral hepatitis in patient

veloped in all patients and a diastolic blood pressure 90 mmHg or more was noted in 4 patients One patient (P A) exhibited significant hypertension 1 month after withdrawing the drug but was normotensive 2 months after cessation of treatment The pretreatment blood pressure of this patient was 138/84 All 5 patients demonstrated glycosuria at some time during therapy but glucose tolerance curves (25 Gm Glucose I V) did not show abnormal levels at 2 hours after injection of glucose Glycosuria ceased promptly with cessation of ACTH administration

A striking symptom upon withdrawal of ACTH was the occurrence of joint pains in 4 of the 5 patients The knees were most commonly involved and in one instance an effusion into the knee joint was confirmed by x ray There was no redness or heat but the joints were tender The pain was severe enough in one patient (J B)

woodwork with white lead paint following this he developed a rash on his hands which lasted for 3 days but there were no gastro intestinal symptoms nor jaundice at that time There was no other recognized exposure to possible hepatotoxins The patient did not drink alcohol The remainder of his personal and past histories was negative

On admission he presented mild jaundice and a few widely scattered acne form lesions of the face and back His temperature was 99 F pulse 60 respira tion 16 and blood pressure 108/64 The heart was not enlarged Regular sinus rhythm was present and the sounds were of good quality A grade I systolic *murmur was heard in the spical and the aortic regions* The lungs were clear to percussion and auscultation The liver did not extend below the costal margin but was slightly tender The spleen was not palpable

Hemoglobin was 12 2 gm WBC 4 050 N 45 (0–4–41) E 4 B 2 M 7 L 42 The urinalysis was normal The cardiolipin test was negative Admission photo fluorogram of the chest was entirely normal

An initial battery of chemical tests of liver function was obtained (Figure 2) and on the 9th day of disease the patient was started on ACTH He experienced only moderate well being while on the medication but his bilirubinemia de clined On the 9th day of drug his heart seemed slightly enlarged and his mur murs had increased in intensity At this time his blood pressure was 135/90 and the neck veins were distended Because of this evidence of cardiac embarrassment medication was stopped He received 825 mg of ACTH in 10 days During the course of treatment this patient maintained a constant weight which proved subsequently to be due to loss of tissue while storing fluid Three days after cessation of ACTH he had diuresed 3 0 kilograms After a 24 hour latent period the patient experienced mild aching in his knees without abnormal physical findings which lasted less than a day

Four days after the end of the treatment course he began to note intermittent symptoms of mild nature similar to those at the onset of his disease anorexia epigastric cramps and later bilirubinuria and acholic stools Five days after stopping ACTH there was an appreciable increase in his serum bilirubin By the end of the second week after stopping ACTH all his tests were more ab normal and bilirubinemia exceeded that on admission At the peak of jaundice his symptoms abated and he then gradually recovered

Serial electrocardiographic tracings failed to disclose significant changes and cardiac fluoroscopy was normal

Case 4 (Figure 1)

R T a 19 year old military policeman was admitted to the Hepatitis Center 23 May 1950 because of jaundice Seven days earlier he had noted epigastric fullness which was rapidly followed by persistent anorexia nausea headache pruritus and dark urine Two days before admission he noticed scleral icterus

Four months prior to entry he was treated for seropositive primary syphilis with 6 000 000 units of procaine penicillin He had had needle punctures 109 and 87 days before the onset of symptoms He had been a heavy beer and whisky drinker for 2 years There was no history of prior jaundice and he had not been exposed to hepatotoxic chemicals

On admission temperature was 98 6 F pulse 54 respiration 18 and blood pressure 1?0/80 There was icterus of skin and sclerae and a minimal chronic acneform eruption overlying the sternum The skin was otherwise clear without telangiectasia The heart and lungs were normal The liver was percussed one

his urine was dark and the next day icterus of the sclerae appeared He had experienced no other symptoms and felt entirely well on admission

The past history included acne and infected sebaceous cysts over a period of 5 years for which penicillin injections had been given 58 and approximately 80 days before the onset of his present illness There were no active lesions at the time of admission to this hospital

He had been in North Africa and the South Pacific from 1944 to 1946 but he had had no tropical diseases There had been no exposure to hepatotoxic chemicals He had never previously been jaundiced He rarely drank alcohol The remainder of the past personal and family histories was not remarkable

On admission the temperature was 99 F pulse 80 respiration 18 and BP 120/80 The skin and sclerae were slightly jaundiced Mild facial and cervical pitting from acne and scattered scars from incisions of cysts were noted There was no telangiectasia Heart and lungs were normal The liver was palpable one fingerbreadth below the costal margin and was slightly tender but the spleen was not palpable The rest of the physical examination was within normal limits

Hgb was 14 8 gms WBC 3 850 with N 52 (0–1–51) L 40 M 3 E 5 Urinalysis was within normal limits and a cardiolipin test was negative Liver function tests demonstrated a total serum bilirubin of 2 2 mgm/100 cc ++ cephalin flocculation 8 thymol turbidity units and 10 gamma globulin turbidity units

The patient started on ACTH 25 mg q i d intramuscularly on the 8th day of his disease On this regimen his appetite and intake were greater than ever before in his life and serum bilirubin concentration fell to normal within 4 days He experienced exceptional well being On the 7th day fullness of the face first appeared and this progressed On the 11th day he began to cough small amounts of frothy white sputum without dyspnea orthopnea or pulmonary rales On the 12th day significant diastolic hypertension developed On the 13th day after 1225 mg of ACTH the drug was stopped when ascites and sacral edema appeared This transiently increased and then disappeared after ten days

The patient had no joint bone or muscle aches in the post therapy period In spite of diuresis his weight did not return to the pre treatment level and his face remained full and rounded It was clinically apparent that he had become fatter probably explained by the continued excellence of his appetite after ACTH Serial electrocardiograms during the period of hospitalization showed no significant changes He was discharged to duty on the 39th hospital day

A recheck examination on the 61st day after the onset of the disease revealed normal findings except for persistent hepatic enlargement with slight tenderness

Case 3 (Figure 2)

A B a 20 year old radio operator was transferred to the Hepatitis Center on 22 May with a diagnosis of viral hepatitis Seven days earlier nausea anorexia dark urine light colored stools chilliness and fever appeared during the course of three days Four days before transfer icterus was noted and he was admitted to the 15th Evacuation Hospital There jaundice and epigastric tenderness without hepatomegaly were noted and his total serum bilirubin was 4 13 mg % On admission to the Hepatitis Center only mild anorexia bilirubinuria and acholic stools persisted

He had had scarlet fever at the age of 7 without remembered sequelae There was no history of acute rheumatic fever Ninety days before the onset of the present illness he had had acute tonsillitis for which repeated aqueous penicillin injections were given Three weeks prior to the onset of hepatitis he had painted

no findings of significance other than sinus bradycardia during the second ACTH period

Case 5

J B a 22 year old tank driver entered the Hepatitis Center on 23 May 1950 because 3 days before friends had noted he had icteric sclerae For the two days prior to entry he had noted dark urine and light stools but had had no other symptoms and felt entirely well

The past history indicated no relevant illnesses He drank 20–25 bottles of beer weekly he had never been in the tropics there had been no exposure to hepatotoxic chemicals he had had no needle punctures in the preceding 6 months Personal and family histories were negative

On examination there was jaundice of skin and sclerae Temperature was 98 F pulse 68 respiration 18 blood pressure 112/68 The heart and lungs were normal The abdomen was flat and muscular The liver could be percussed 2 finger breadths below the right costal margin but could not be felt The rest of the physical examination was within normal limits

Hgb was 13 2 gm % and WBC 4 350 N 57 (0–14–43) L 24 M 7 Bl Eos 11 Urinalysis was within normal limits as was a chest X ray Laboratory tests of liver function revealed a total serum bilirubin concentration of 4 9 mgm/100 cc negative cephalin flocculation 3 75 thymol turbidity units and 6 25 gamma globulin turbidity units

On the fifth day of disease ACTH was begun The patient received a total of 975 mg in 11 days during which time he felt extremely well His appetite was enormous and he ate far more than ever before in his life Serum bilirubin concentration fell rapidly and was normal within 9 days of treatment. Hypertension a plethoric rounded facies and sacral edema were associated with drug administration Engorged neck veins and a soft blowing pulmonic systolic murmur were first noted after the tenth day of drug Ascites appeared on the eleventh day of ACTH and the medication was stopped

Approximately 16 hours after his last dose the patient suffered excruciating pains in his legs which started at the dorsa of his feet and radiated to his knees 24 hours later an unequivocal small effusion was detected in the left knee joint which was confirmed by X ray examination The right knee was slightly less tender but no definite signs of effusion existed there Neither extremity demonstrated local heat or redness and pain was not greatly increased by motion In the first 24 hours of pain a total of 2 1 gm aspirin and 0 18 gm codeine were ineffective and the patient required two parenteral 100 mgm doses of demerol for relief

A minimal acneform eruption limited to the anterior central thoracic region first appeared on the day following cessation of ACTH

To elucidate further the relation of joint pains to the ACTH treatment in this patient the drug was reinstituted in the same dosage schedule 53 hours after cessation of the first course Four and one half hours later when his eosinophile count was reduced by 46% his pain had not remitted For 24 hours after the drug was restarted his pain was still severe but thereafter it improved the effusion disappeared and by the 49th hour he had again become asymptomatic with no evidence of joint disease on examination On the second day after re institution of therapy his appetite returned to that level which prevailed during the first course his face became more plethoric and fatter than it was in the short time off the drug ascites began to increase and he again experienced

fingerbreadth below the right costal margin but an edge was not felt The spleen was not felt The rest of the physical examination was within normal limits

Hgb was 14 8 gms WBC 4150 with N 50 (0–6–44) L 37 M 8 E 4 B 1 Urinalysis was within normal limits and a cardiolipin test was negative

Two sets of liver function tests were obtained before starting ACTH on the 4th hospital day Four days later anorexia had disappeared and his intake was enormous Pruritus ceased on the 6th day of drug Intermittent midabdominal pains and hepatic tenderness continued The fall in his bilirubin during this period is shown in Figure 1 During the latter days of ACTH administration a pulmonic systolic murmur rounded full facies and sacral edema appeared

On the 12th day of ACTH because of the development of ascites medication was stopped Within 14 hours arthralgia appeared in his wrists elbows ankles and knees without effusion or other local finding except tenderness Twenty four hours later the pain encompassed the whole of his lower extremities and the left shoulder girdle with tenderness of muscle bone and joint The pain in these regions was not changed during movement but was considerably increased in the post activity period After a total of 80 hours these extremity pains dis appeared

The second day after the patient was off ACTH a small right sided epistaxis occurred

Approximately 48 hours after cessation of ACTH the patient began to ex perience his original symptoms again Pruritus anorexia nausea vomiting leth argy and epigastric cramps became prominent and a striking increase in his total serum bilirubin from 2 02 to 16 70 mg % occurred in seven days During this period a marked diuresis occurred (7 3 kg weight loss) and the rounded face reverted to normal His indolent anterior thoracic acne became active the day after withdrawing ACTH and a florid acneform eruption appeared and steadily worsened during the days off the drug On the 7th day off medication he appeared acutely ill He was deeply jaundiced and suffered severe pruritus Innumerable petechial hemorrhages appeared in the areas where he had scratched A tourni quet test was negative after 3 minutes Bleeding time was 2 minutes venous clotting time 5 minutes (Lee White method) and platelet count was 127 680

On the seventh day after cessation of ACTH therapy because of his severe relapse ACTH was restarted in the same dosage as before Within 6 hours nausea and abdominal discomfort vanished his appetite became better and he felt generally improved By the 72nd hour he was energetic and cheerful his appetite was enormous and his total serum bilirubin had fallen to 10 40 mg % a drop of 6 30 mg % During therapy pruritus slowly disappeared and his acne healed Mild purpura in scratched areas continued to appear for seven days and small right epistaxes occurred on the 8th and 9th days of the second course of treatment Moderate tenderness of the lower thirds of both tibiae was present from the 7th day of therapy until cessation of ACTH Variable sinus bradycardia to a recorded low of 38 appeared in the latter half of the therapeutic trial without other physical or electrocardiographic abnormality Rapidly accumulating ascites and mild somnolence on the 9th and 10th days prompted discontinuation of the drug On the tenth day his total serum bilirubin had fallen to 1 68 mg %

Immediately after cessation of treatment a similar but milder relapse ensued Pruritus and jaundice (maximum serum bilirubin 3 01 mg %) again occurred The hyperbilirubinemia slowly declined over a 16 day period His subsequent course was one of slow convalescence with elevated bromsulphalein retention values

Serial electrocardiographic tracings throughout the hospitalization revealed

by the inciting agent itself (ACTH) but then appearing with drug withdrawal

SUMMARY

1 5 patients with acute viral hepatitis received 100 mgm of ACTH daily in 4 divided doses for periods ranging between 9 and 21 days Marked symptomatic improvement occurred promptly and was associated in all instances with a falling serum bilirubin con centration

2 Upon withdrawal of ACTH arthralgia developed in 4 patients one of whom demonstrated joint effusion

3 Other untoward effects seen were glycosuria hypertension edema ascites moon face and acne

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DISCUSSION

DR V M SBOROV (Army Medical School Washington) One year ago at the first ACTH Conference brief mention was made of the treat ment of two cases of acute viral hepatitis with ACTH by the Hepatic and Metabolic study group of the Army Since that time this experi

exceptional well being The chest eruption increased greatly during the second course A small unilateral epistaxis occurred on the 4th day of treatment

After 675 mg given in 8 days the drug was again stopped since at that time there was no clinical or laboratory evidence of hepatitis 24 hours later a definite small effusion was present in the right knee with mild pain but his other bones and joints were entirely symptom free The effusion was no longer detectable at 24 hours but mild pain persisted gradually involving the left knee until the 4th post treatment day when severe pain again appeared in the right knee without effusion This lasted 22 hours more and then disappeared completely

The anterior thoracic acne which had become marked during the second course of treatment progressively involuted after stopping medication On the second post treatment day a mild folliculitis appeared at the hair line which persisted for two days The plethoric full facies gradually regressed after cessation of ACTH

Hepatic function tests showed a progressive return to normal without evidence of relapse

Serial electrocardiograms during the period of hospitalization showed no significant changes

COMMENT

The apparently beneficial result produced by ACTH in the treatment of acute viral hepatitis in this study is in agreement with the finding of Thorn et al [2] in the treatment of a case of homologous serum hepatitis However the present report is at variance with that of Bluemle et al [6] in which no improvement attributable to ACTH was noted in two cases of acute viral hepatitis In our investigation good results were seen in all five cases It is not our intention to assign the recovery of some of these patients to ACTH administration but the course of disease in the patient R T where remission was associated with ACTH administration and relapse withdrawal on two occasions points to a beneficial effect of ACTH therapy in acute viral hepatitis

The occurrence of migrating arthralgia in 4 of the patients concomitant with hormone withdrawal requires comment The abrupt discontinuance of ACTH was most probably followed by a period of adrenal insufficiency and during this period the joint and muscle pain developed The diuresis occurring during a time of relative adrenocortical hypofunction may have produced a sodium loss which resulted in muscle cramps but the presence of joint effusion in patient J B suggested that more than electrolyte imbalance was needed to explain the symptoms One of the patients (A B) probably had a pre existing rheumatic diathesis but the other 3 patients who presented symptoms of arthralgia exhibited no evidence from history or physical examination of rheumatic fever or rheumatoid arthritis

Serum sickness should be mentioned as a possible cause of the arthralgia The development of symptoms could have been masked

course (Fig 4) The other liver function tests showed a parallel drop to normal at a faster rate than did the controls (Fig 5)

Liver biopsies were taken in nine of these treated cases on the day therapy was begun and on the day following the completion of therapy A comparison of these biopsies showed a marked diminution with a localization of the cellular exudate following the course of ACTH (Figs 6 and 7) Since biopsies were not taken at correspond

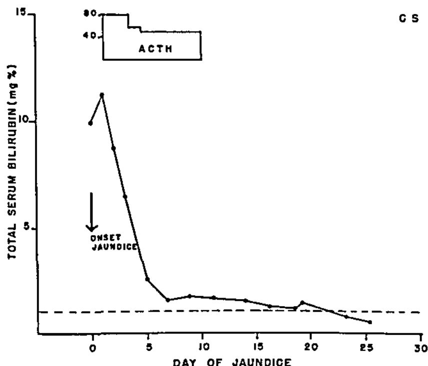


FIG 3 Treatment with ACTH started on second day of jaundice Sharp drop of serum bilirubin followed by a leveling off of curve of fall Normal value reached 22 days after onset of jaundice

ing intervals in the control cases it is not known whether this diminution of inflammation is related to therapy alone

It is the general impression of our attending staff that the results obtained in the two cases treated with a slow infusion of ACTH intravenously are better than those with the single dose subcutaneous method This was true despite the fact that with the latter course a much smaller total dosage was used It appeared that the appetite general well being and liver function tests returned to normal at a significantly faster rate than did those in the first group treated The eosinophil drop likewise was more complete in the cases treated with

ence has grown and now includes fourteen treated cases Treatment in all of these cases was begun in the first week of jaundice After diagnosis the patients were followed at frequent intervals by physical examination and a panel of liver function tests This panel included serum bilirubin one minute and total cephalin-cholesterol flocculation thymol turbidity prothrombin time serum cholinesterase Kunkel gamma globulin urine bilirubin and urine urobilinogen Bromsulfthalein (BSP) retention was measured following the return to normal of the serum bilirubin

In the first group consisting of twelve cases *ACTH was administered in a single dose daily* A total of 520 milligrams was administered over a nine day period Eighty milligrams were injected subcutaneously for the first two days Sixty milligrams were given the third day followed by fifty milligrams daily for six days

In two cases a total of 300 milligrams was administered intravenously over a ten day period This was divided into forty milligrams daily for the first five days followed by twenty milligrams daily for the last five days The hormone was diluted in 1000 cc of five percent glucose and dripped intravenously very slowly over a ten to twelve hour period In all cases response was measured by a drop in eosinophils

In addition to the ACTH all patients were treated uniformly with bed rest until the total serum bilirubin returned to one milligram or less A high protein high caloric diet was offered and the patients were encouraged to eat second helpings

A group of eighteen controls was selected and followed in a similar manner With the exception of the ACTH these patients received treatment identical with that in the first group It should be emphasized however that these two groups may not be strictly comparable since they all represented sporadic cases The patients in both groups undoubtedly included both virus IH and virus SH types

By all standards of comparison used in the treated and control cases the treated cases generally showed a more favorable response Shortly following the beginning of therapy there was noted a sharp drop in the serum bilirubin in the majority of cases This was followed by a more gentle drop in the curve of fall which ultimately reached normal in an average of twenty seven plus* days (11 cases) (Fig 3) This compared to an average duration of jaundice in the control cases of fifty days A few of our cases demonstrated a continued rise in *the level of serum bilirubin for a few days preceding* the characteristic sharp drop This was apparently related to the fact that these cases were started on therapy exceptionally early in their

* Four cases were included in this average whose total serum bilirubin was not yet normal In all of these cases the total serum bilirubin was less than 2 mgms



Fig 6 Case C S Liver biopsy prior to therapy Section shows increased cellular infiltrate characteristic of acute viral hepatitis

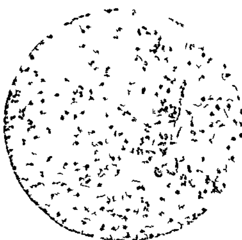


Fig 7 Case C S Liver biopsy 9 days after start of therapy Section shows clearing of inflammatory exudate

DR POINTS (Minnesota) I would like to know whether bilirubin excretion was studied in any of these patients? It seems an excellent place to find out how much bile pigment is stored and how rapidly

DR A E EISENSTEIN (Barnes Hospital and Washington University School of Medicine St Louis) I would like to ask a question Would he care to comment on the liver function tests other than the serum bilirubin?

DR JAMES W COLBERT JR The influence of ACTH upon the reaction of the cephalin flocculation and thymol turbidity tests in our cases was not clear In the absence of clear-cut results the small number of patients did not permit any comparison with the four thousand patients who had been hospitalized at the Hepatitis Center Again 24 hour urine assays of urobilinogen did not reveal consistent results

Whether or not jaundice adversely affects ACTH therapy I am not prepared to say However it should be stated that since this study with ACTH in the treatment of viral hepatitis we have had the opportunity to treat two patients who were profoundly ill at the beginning of ACTH administration Both of these patients continued a downhill course and in spite of the administration of what appeared to be adequate amounts of hormone both patients died

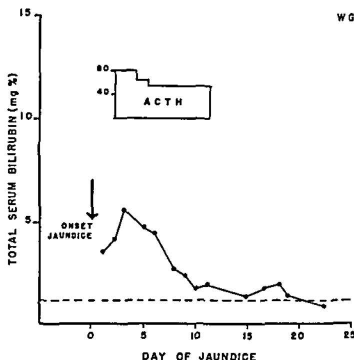


Fig 4 Rise of serum bilirubin in first few days of therapy followed by a gradual fall Normal value 20 days after onset of jaundice

ACTH THERAPY IN ACUTE VIRAL HEPATITIS

C mp f the ge d t f b m l l er f est th t e t d d co l g p F g d t h mb f d ys fr m th s t f j d to be f t m f l e

	BILIRUBINURIA	ELEVATED TOTAL SERUM BILIRUBIN	ELEVATED THYMOL TURBIDITY	ELEVATED CEPH CHOL FLOC
CONTROLS	20 (18)	50 (18 as)	53 (12)	47 (13)
TREATED	10 (13)	27 pt (11)	14 (9)	15 (8 as)

Fig 5 ACTH therapy in acute viral hepatitis (Table)

intravenous ACTH Although a few side reactions were noted it is our belief that in no instance did the ACTH adversely affect the course of hepatitis

DR EMANUEL B SCHOENBACH We treated a group of patients with Hodgkin s disease with ACTH and cortisone and noted that there were four patients who did not show any response to cortisone or ACTH The patients were jaundiced secondary to Hodgkin s infiltration of the liver I was just wondering how that correlates with your observations in hepatitis

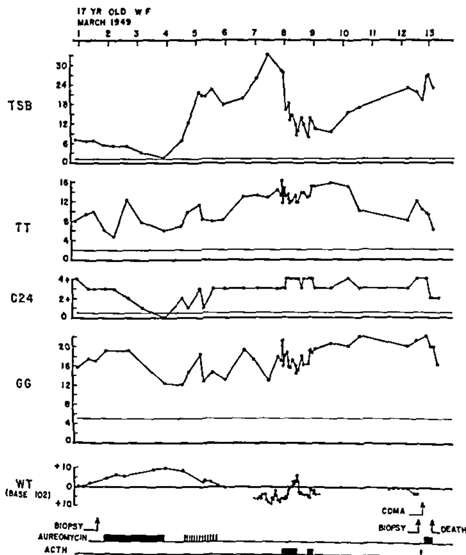


FIG 1

adequately studied in this regard Thymol turbidity was reduced significantly in 4 cases as was Kunkel gamma globulin A temporary increase in urinary coproporphyrin excretion of from 5 to 14% of the pre treatment values occurred in each of the 2 patients in which this was studied A reduction in bromsulphthalein retention of from 5 to 6% of the amount of dye injected occurred in each of the 2 pa

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The Treatment of Chronic Inflammatory Liver Disease with ACTH and Cortisone

Paul Gyorgy and L W Bluemle, Jr

HOSPITAL OF THE UNIVERSITY OF PENNSYLVANIA UNIVERSITY OF PENNSYLVANIA MEDICAL SCHOOL PHILADELPHIA AND VALLEY FORGE GENERAL HOSPITAL PHOENIXVILLE PENNSYLVANIA

Five patients with chronic liver disease with at least six months duration were treated with cortisone and one with ACTH The case treated with ACTH was reported preliminarily at the First ACTH Conference when we noted immediate improvement as reflected by increased appetite amelioration of symptoms and a precipitous decline in serum bilirubin Fig 1 During the second week of therapy however she developed rapidly increasing ascites and edema which necessitated discontinuation of ACTH Unfortunately she had not been maintained on a low sodium diet which may have minimized fluid retention Following withdrawal of ACTH a prompt diuresis ensued and 12 days later a second course of ACTH was given By this time there was little evidence of improvement One month later the patient s condition became gradually worse and she died in hepatic coma shortly after the onset of a 3 day course of ACTH It is interesting to note that her first signs of mental improvement occurred after the second 20 mg dose of ACTH

Of the 5 patients with chronic hepatitis treated with cortisone 2 were thought to have been of viral origin 1 of possible amebic origin and 2 resembling the cholangiolytic type of chronic hepatitis described by Watson and Hoffbauer Dosage ranged from 600 mgs over 6 days to 2800 mgs in 3 courses over 6 months as shown in Fig 2

Symptoms were ameliorated in 2 slightly worsened in 1 and unchanged in 2 who were relatively asymptomatic prior to therapy An increase of from 2 to 3 cm in liver size was observed in 2 Serum bilirubin decreased from 0 3 to 3 1 mgs /100 ml in all 5 cases the greater reduction occurring in those cases with elevated serum bilirubin Increases in urinary urobilinogen excretion varying from 50 to 500% of the pre treatment values were found in all of the cases

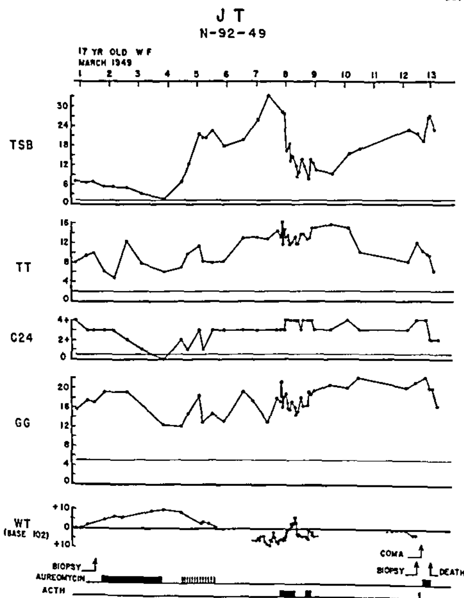


FIG 1

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tients in whom this test was performed during therapy Post treat ment liver biopsies in 4 cases revealed liver glycogen content in 2 associated with less inflammatory cell infiltration but increased fibrosis in one There were no significant changes in the other 2 cases

Numerous other biochemical data were obtained during this study most of which are excluded from this presentation because of the relatively insignificant or inconsistent variations that were noted during the administration of ACTH or cortisone In this category are included cephalin cholesterol flocculation alkaline phosphatase prothrombin time serum cholinesterase and serum globulin Serial

EFFECTS OF ACTH AND CORTISONE IN CHRONIC HEPATITIS

	ACTH (1)	CORTISONE (5)
SYMPTOMS	VARIABLE	AMELIORATED (2) WORSENED (1)
PHYSICAL FINDINGS	NO CHANGE	INCREASED LIVER SIZE (2)
S BILIRUBIN	PRECIPITOUS DECREASE	SLIGHT DECREASE (5)
U UROBILINOGEN	NO CHANGE	INCREASE (4)
THYMOL TURBIDITY	SLIGHT DECREASE	SLIGHT DECREASE (4)
S ALBUMIN	NO CHANGE	VARIABLE
K GAMMA GLOBULIN	SLIGHT DECREASE	SLIGHT DECREASE (4)
S CHOLESTEROL	SLIGHT INCREASE	VARIABLE
S CHOLESTEROL ESTERS	SLIGHT INCREASE	VARIABLE
BSP RETENTION	———	DECREASE (2)
U COPORPHYRIN	———	TEMPORARY INCREASE (2)

Fig 2

oral glucose tolerance tests indicated temporarily decreased glucose tolerance in 4 cases receiving cortisone

Urinary excretion of 17 ketosteroids was reduced from 12 to 28% below pre treatment levels in 4 cases during cortisone administration while no consistent effect was noted on excretion of urinary corticoids Serum gamma globulin as determined electrophoretically was slightly reduced in 2 out of 3 cases receiving cortisone in which this protein was determined

Regarding possible mechanisms of these apparent effects of ACTH and cortisone in chronic liver disease we know relatively little Possibly the subjective improvement noted in some cases may be attributed in part at least to nonspecific effects rather than to a direct effect on the liver disease alone

What influence ACTH and cortisone exert on the function of a diseased liver as determined by changes in results of biochemical tests must also be assessed cautiously It is conceivable that many of the changes observed in this study could have been mediated through mechanisms not entirely dependent on hepatic function Hemodilution consequent to fluid retention may have been partially responsible for the observed temporary reduction in concentration of certain serum constituents such as bilirubin although a corresponding fall in *hematocrit level does not* bear this out *The temporary elevation* of urinary urobilinogen associated with a decrease of serum bilirubin in 5 cases suggests some improvement in pigment metabolism Reduction of bromsulphthalein retention in all cases tested would also suggest this Unfortunately we have no data on fecal urobilinogen changes

SUMMARY

In summary we can conclude from this very limited experience that ACTH and cortisone appear to be of at least temporary value in certain cases as therapeutic adjuncts in the management of chronic liver disease They have proven particularly useful in relieving anorexia and thus allowing better nutrition We have not determined by what other criteria beneficial results may be predicted Nor have we learned as yet the optimum dosage schedule We believe that it may be preferable to give short courses of either ACTH or cortisone at intervals of one or two months along with of course other therapeutic measures We would welcome any suggestions you might have in this regard or in the direction of future efforts to appraise the uesfulness of these drugs in chronic liver disease

DISCUSSION

See discussion on following paper

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Observations During the Administration of ACTH to Patients with Chronic Liver Disease

Alfred M Bongiovanni and William J Eisenmenger*
HOSPITAL OF THE ROCKEFELLER INSTITUTE NEW YORK

Adrenocorticotrophic hormone was administered to a group of patients with chronic hepatic disease most of whom had far advanced cirrhosis These were of three types (1) so-called Laennec s cirrhosis (2) biliary cirrhosis of the xanthomatous type (3) cirrhosis of undetermined etiology confined to young females The latter group consists of a number of patients with a syndrome not previously definitively delineated restricted to young females under 35 years of age with jaundice of insidious onset and a progressive course toward advanced cirrhosis and hepatic insufficiency The characteristic signs which were associated with the earliest manifestations of liver disease included hirsutism pigmented abdominal striae obesity acne amenorrhea and moon facies These did not appear to occur secondary to chronic hepatic dysfunction and in our experience have not been observed in such combination in late cirrhosis of defined etiology Finally the patients in this category have been found to excrete large quantities of glycogenic corticoids in their urine This group demonstrated interesting changes with the administration of adrenocorticotrophin to be described The clinical features of the last category have been detailed elsewhere [1,2]

All patients were treated with 100 mg adrenocorticotrophin daily for periods of 6–12 days Six patients so treated were carefully followed by multiple serial laboratory studies Particular attention is focused on a presentation of the latter belonging to each of the three types of cirrhosis indicated

The urinary excretion of 17 ketosteroids was initially depressed to a great degree in five of the six patients (Table I) With the administration of ACTH the urinary levels of 17 ketosteroids rose quickly having increased by 100 per cent or more within 18 hours This was

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Table I

17 KETOSTEROID EXCRETION IN CIRRHOSIS

Pt	Sex Age	Range mg per 24 hrs	Avge	Normal avge for age sex
VV	M64	3 1– 6 9	5 0	9 0
MS	F16	7 0–11 5	9 2	9 0
DM	F19	3 9– 7 0	5 3	10 2
LB	F50	3 8– 4 2	4 0	8 6
MH	F46	3 5– 9 4	7 0	9 3
LG	F50	5 1	5 1	8 6

maintained throughout the period of administration falling to the initially low levels (or lower) within 48 hours of termination The excretion of reducing corticoids determined by the method of Heard and Sobel rose in most cases exceeding 100 per cent of the initial values on the fourth or fifth day of administration of ACTH These phenomena are illustrated in two representative cases in Figure 1 It may therefore be concluded that there is little if any limitation in the response of adrenal steroid production as measured by the urinary excretion to adrenocorticotrophin in these patients with chronic hepatic disease even of advanced degree This observation contradicts Conn s suggestion[3] that such might not occur in liver disease with low serum cholesterol esters Figure 1 also illustrates a rise in

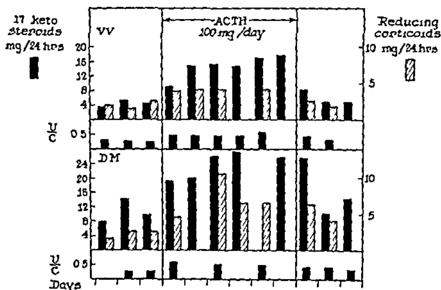


FIG 1

the uric acid/creatinine ratio of 50 per cent or more with the administration of the hormone

The total number of eosinophiles in the circulating blood of patients with cirrhosis is somewhat depressed This may be explained in terms of the increased level of reducing corticoids reported[4] in the urine of these patients Following the administration of 25 mg ACTH the number dropped to zero in every instance tested (Figure 2) This is in accord with the above data indicating an adequate response of the adrenal cortex to ACTH in patients with cirrhosis The response was less marked with epinephrine

Separation of the urinary 17 ketosteroids into alpha and beta

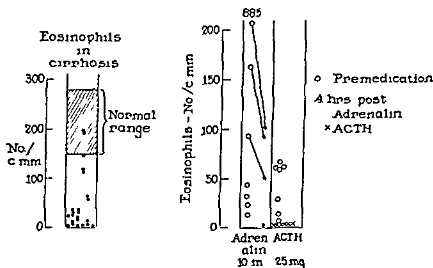


Fig 2

fractions indicated that the initial depression in urinary excretion occurred mostly in the alpha stereoisomer (Table II) After administering ACTH the rise in the urinary 17 ketosteroids occurred almost entirely in the alpha fraction in a single patient so studied

By use of the enzyme glucuronidase prepared from bacterial sources it was found that 50 per cent of urinary 17 ketosteroids were excreted as glucuronidates in normal individuals (Table III) However only 20 per cent or usually much less was conjugated as glucuronidates in the urines of patients with cirrhosis and low levels of excretion In the light of the role of the liver in glucuronide synthesis[5] it appears that such a low proportion of these steroids occurring in the urine as glucuronidates may be attributed to hepatic damage This proportion was often zero It is therefore believed that the low excretion of these steroids in chronic liver disease may be due at

Table II

17 Ketosteroid Fractions (mg /24 hrs)

Pt	*Sex*	*Total*	*Non Ketonic*	*Alpha Ketonic*	*Beta Ketonic*	*% Alpha*	*% Beta*
AL	M	9 8	0 62	6 7	2 5	73 0	27 0
RM	M	5 5	0 18	4 0	1 3	68 0	25 7
AS	M	5 1	0 75	4 4	0	85 0	
MS	F						
(after ACTH) 19 3			2 60	16 7	0	86 5	

Table III

17 Ketosteroids Released as Glucuronidates

Patient	*Hydrolysis*			
	Acid m s	*Glucuronidase*		*None*
		mgs	*%*	
ND	4 5	0 9	20 0	0
MR	4 3	0 5	12 0	0
(conv)	6 2	1 9	31 0	0
AL	7 3	0 2	3 0	0
(conv)	12 0	6 7	56 0	0
DM	3 9	0	0	0
(ACTH 1st day)	9 5	2 1	22 0	0 1
(2nd day)	12 8	3 5	27 0	0 1
CG	4 8	0 9	19 0	0
LB	1 6	0 2	12 5	0
3 Normals			45–55	

least in part to an inability to convert them into water soluble hence urine disposable compounds and not entirely to adrenal hypofunction Following the administration of ACTH serial determinations in patient D M (Table III) indicate a rise in total neutral 17 ketosteroids and a remarkable increase in the percentage of steroids excreted as glucuronidates Such a rise in the percentage of conjugated steroids is also apparent in A L during convalescence several months after the initial observations This suggests that ACTH in some way increases the ability of the liver to conjugate these compounds This may be related to the glycogenic function of certain of the adrenal corticoids produced in greater quantity in response to adrenocorticotrophin This effect was sustained only so long as ACTH was administered

Fasting blood glucose levels were determined daily in seven patients throughout the experiment (Figure 3) Significant rises (above 150 mg per cent) from previously normal levels were seen in three patients This resulted during short term therapy and in fact always occurred before the third day This is a higher proportion of such responses of fasting blood glucose levels than has been seen during the use of ACTH in many other conditions Alterations in glucose

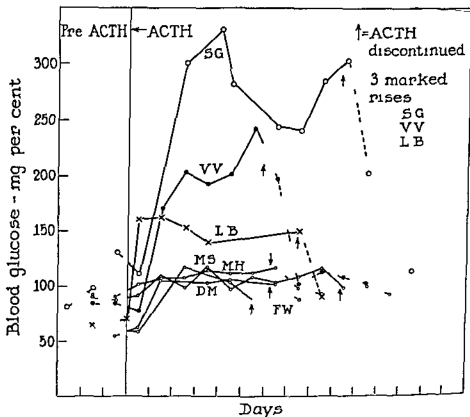


FIG 3

tolerance and glycosuria have been more frequently encountered but the present observations in relation to fasting levels in this group of *far advanced cirrhotics* appear significant In none was there reason to suspect a pre diabetic state It may be that a severely diseased liver is not able to accommodate the large quantities of carbohydrate produced by the glycogenic hormones of the adrenal resulting in elevation of the blood glucose All levels fell immediately upon cessation of therapy Alternately it may be a reflection of the possible inability of the liver to metabolize glycocorticoids

The standard liver function tests failed to reveal substantial im

provement or significant changes in ten of the more clearly defined cirrhotic patients studied However in the group of patients with cirrhosis of undetermined etiology the young females in the category defined above notable changes occurred which were accompanied by clinical amelioration Two of this group are of the present series and two others (not herein described) indicated the following re sponses This newly defined syndrome was generally characterized by a low level of albumin in the serum but a high total protein often above 10 0 gms per cent due to elevations of the gamma glob

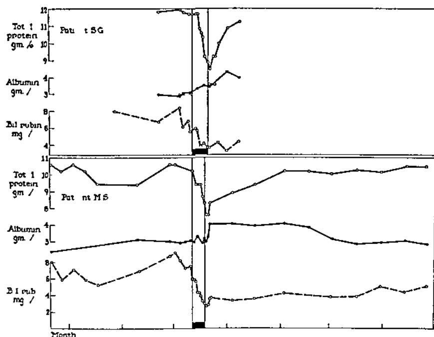


FIG 4

ulin Following only six days treatment with ACTH 100 mg daily there occurred in these patients a rapid drop in the level of total protein accompanied by a rise in serum albumin thus restoring the blood protein pattern to normal (Figure 4) In addition the serum bilirubin which was characteristically elevated diminished con siderably in concentration The alterations in protein were demon strated by four techniques including electrophoretic analysis These changes persisted well after discontinuation of therapy but were not permanent since the protein pattern reverted several weeks later However a repetition of the course of ACTH again brought about the described changes in protein pattern There was subjective

clinical improvement in each instance Long term therapy of this single group of patients is presently being undertaken in an attempt to alter the course of an otherwise progressive disease

Certain changes of interest occurred in the blood clotting mecha nism which have not yet been elucidated In six patients with bleed ing gums which had persisted in spite of classical forms of treatment there was complete relief of this complaint within 24 hours of initiat ing ACTH therapy Three patients suffered from acute abdominal pain increasing ascites or the occurrence of ascites for the first time and intra abdominal hemorrhage on the third to the sixth day This was believed to be due to portal thrombosis and in fact was confirmed in one patient who died shortly after such an episode In an addi tional patient peritonitis supervened in the face of these symptoms

Table IV

EFFECTS OF ACTH ON SERUM LIPIDS OF PATIENTS WITH LOW INITIAL VALUES AND CIRRHOSIS OF UNDETERMINED ETIOLOGY

Patient MS	*Date*	*Total Cholesterol mg %*	*Total Phospholipid mg %*	*Total Lipid mg %*
Pre treatment	12–9–49	112	181	487
ACTH (100 mg daily)	12–19–49	134	213	608
	12–22–49	150	290	690
After treatment	1–4–50	171	345	800
	1–7–50	156	280	695

To date insignificant and slight changes have been noted in pro thrombin time and antithrombin titers which could favor coagula tion However we believe that an uninvestigated factor is responsible for these apparent changes in blood clotting These changes super imposed upon a relative portal stasis may be responsible for portal thrombosis In our experience this has been a limiting factor in the use of ACTH in chronic liver disease of advanced degree

During the use of ACTH there was no rise in the urinary ex cretion of creatine with one exception The sodium concentrations in urine saliva and sweat which were already low in cirrhosis fell farther The plasma volumes as determined with the dye T 1824 rose 10–20 per cent The latter did not detract from the protein changes described in the group with cirrhosis of undetermined etiol ogy (Figure 4) since these persisted and the rise in albumin became more apparent after the plasma volume returned to normal

The effects of ACTH on the serum lipids of a single patient with cirrhosis of undetermined etiology and low initial values are illus

trated in Table IV A rise in all components studied is noted similar to that reported following the use of ACTH in other conditions [6]

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DISCUSSION

DR PETER H FORSHAM The syndrome of these young women looks very much like an aborted form of Cushing s syndrome One wonders which comes first the Cushing s syndrome or the liver disease One might assume that if the liver disease came first the destruction of corticoids would be diminished and that such a state might end up with Cushing s syndrome Do these patients respond better to ACTH and show a moon face more quickly than did the other cirrhotics?

One wonders what is there about these young women that would predispose them to Cushing s syndrome for a given level of corticoids?

DR LAURANCE W KINSELL Just one observation in regard to Dr Bongiovanni s thought about the role of the ability of the liver to store glycogen in relation to the hyperglycemia which can be produced during ACTH administration In one individual with a major hepatic congestion on the basis of primary cardiac failure the initial administration of ACTH caused a very great elevation of blood sugar The subsequent control of the hepatic congestion by the use of an essentially zero sodium intake and a high protein intake (with the use of a desalted milk preparation) resulted in an essentially complete disappearance of the hyperglycemia and in association with this the complete disappearance of the hepatomegaly The inability of the liver to store glycogen therefore can probably be a very real predispositioning factor in the production of hyperglycemia

DR HAROLD BROWN (Salt Lake General Hospital and University of Utah School of Medicine Salt Lake City) We have some pertinent data on four alcoholics who were treated with ACTH for their cirrhosis of the liver in doses of 70 to 200 mg per day for periods of 10 to 15 days

We might say first of all that the most striking effect was on the appetite All of these patients had a marked increase in appetite

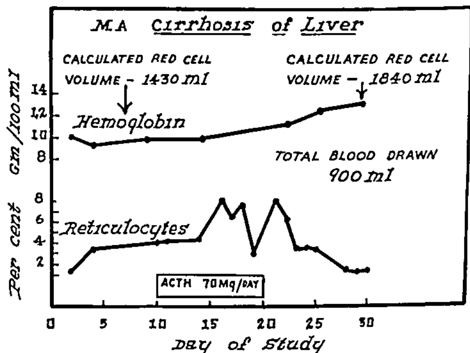


FIG 5 Effect of ACTH on erythropoiesis in a case of cirrhosis of the liver The red cell volume was calculated from the plasma volume and hematocrit values

which was noted on the first day of therapy and which tended to persist even after the cessation of therapy

The drug also seemed to have some effect on the anemia which these four patients had to a moderate degree During the course of our studies about one liter of blood was drawn from each patient over the course of about a month and it is interesting that the hematocrit was as high or higher at the end of the month than it had been before In two patients there was a significant rise in hemoglobin and in one (figure 5) the hemoglobin and hematocrit rise was associated with a reticulocytosis at the time the ACTH therapy was given This was not due to fluid shifts because we calculated the plasma volumes in these

patients and from that calculated the total red cell volumes which rose in the patient described from 1430 ml to 1840 ml

We were also interested in whether ACTH did have a deleterious effect on the liver because there is a fair amount of literature indicating that ACTH or cortisone might cause fatty infiltration of the liver

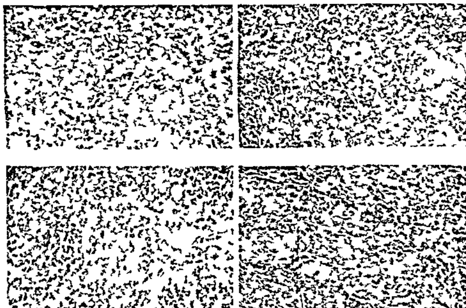


FIG 6 Histological appearance of liver before and after ACTH therapy *Upper Left* Section of liver biopsy of patient M A taken on 5 June after 10 days of a high protein diet *Upper Right* Section of liver of patient M A on 15 June after patient had received 70 mgms of ACTH daily for 7 days *Lower Left* Section of liver biopsy of patient R D taken on 23 October after 14 days of a high protein diet *Lower Right* Section of liver taken on 13 November after the patient R D had received 150 to 200 mgms of ACTH daily for 14 days In both these patients ACTH treatment was associated with a clearing of the fat and inflammatory reaction in the liver

One patient with cirrhosis and a fatty liver showed marked clearing of fat during the period ACTH was given and he was receiving 150–200 milligrams a day In figure 6 are shown liver biopsies before and after ACTH therapy in two patients The patient I have mentioned is in the lower portion of the slide Another patient who had cirrhosis as well as fatty and cellular infiltration had been on a high protein diet for 10 days at the time the first biopsy was taken and then had been on ACTH for seven days at the time the second biopsy

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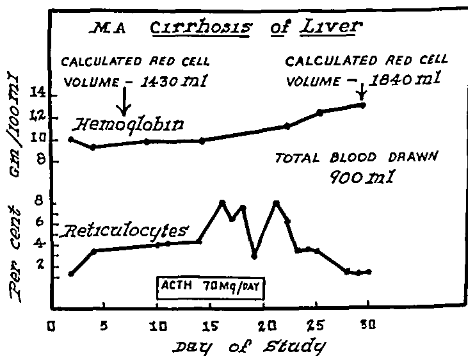


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sulphthalein retention of from 6 to 7% of the dye administered in all cases receiving cortisone

Post treatment liver biopsies in the ACTH liver treated group when compared with pre treatment biopsies showed less periportal fibrosis and less fatty vacuolization in the one patient whose liver was markedly reduced in size and no apparent change in the other two In the cortisone treated group post treatment liver biopsies revealed decreased fat content in 1 increased glycogen content in 2 and no apparent change in 1 No apparent advantage was gained by combining ACTH therapy and cortisone therapy in the 1 case so treated

EFFECTS OF ACTH AND CORTISONE IN LAENNEC S CIRRHOSIS

	ACTH (3)	CORTISONE (3)
SYMPTOMS	VARIABLE	NO CHANGE (3)
PHYSICAL FINDINGS	DECREASED L ER & SPLEEN SIZE (1) DECREASED SPLEEN SIZE (1)	NO C A GE (2) INCREASED LIVER SIZE (1)
S BILIRUBIN	SLIGHT DECREASE (2)	SLIGHT DECREASE (3)
U UROBILINOGEN	VARIABLE	VARIABLE
THYMOL TURBIDITY	VARIABLE	VARIABLE
S ALBUMIN	VARIABLE	VARIABLE
K GAMMA GLOBULIN	DECREASE (2)	VARIABLE
S CHOLESTEROL	VARIABLE	INCREASE (3)
S CHOLESTEROL ESTERS	INCREASE (2)	SLIGHT DECREASE (3)
BSP RETENTION	TEMPORARY DECREASE (2)	DECREASE (3)
U COPROPORPHYRIN	———	INCREASE (3)

FIG 7

DR EDMUND FLINK (University Hospital Minneapolis) We have treated 9 cases of hepatic cirrhosis and 1 case of Hodgkin s disease with pruritus with ACTH for periods varying from 6 to 12 days Three of these patients had cholangiolitic cirrhosis with pruritus of months or years duration One had biliary cirrhosis and the remainder were Laennec s cirrhosis

In the first 4 patients noted above who had elevated blood bile acids and pruritus there was a decrease of varying degree in the blood bile acids (Fig 8) and a decrease or a disappearance of pruritus during ACTH administration The patient with Hodgkin s disease (L Fig 8) had some decrease in bile acid but no change in the pruritus

was taken The second biopsy shows clearing of fat and inflammation cells

It is well realized that one may select proper sections for photo graphic purposes and give about any impression that he desires but these are fairly representative sections of the liver biopsies

In two other patients with fibrosis of the liver in whom biopsies were taken no change could be demonstrated after ACTH treatment

ACTH has been recommended by some for the treatment of de lirium tremens and we have had one patient who had been in delir ium tremens on several occasions—so that we were familiar with his pattern—and he had a typical attack on the tenth day of ACTH treat ment This episode has caused some doubt in our mind as to the efficacy of ACTH in the treatment of delirium tremens

DR PAUL GYORGY At the first Clinical ACTH Conference we re ported 3 cases of chronic liver disease treated with ACTH Since that time we have studied 5 patients with Laennec s cirrhosis two with ACTH alone two with cortisone and one with a course of ACTH followed in ten days by a course of cortisone

The average dose of ACTH varied from 500 to 1 000 mgs over a period ranging from nine to ten days The total dosage of cortisone varied from 800 to 1 000 mgs over a period ranging from six to ten days

Fig 7 is a summary of our observations in this group of cirrhosis of the liver cases The symptomatic response to ACTH was variable One patient experienced dramatic subjective improvement mani fested by an increased appetite permanent weight gain and increased exercise tolerance The other two patients who were relatively asymp tomatic prior to therapy noted little if any change A significant de crease in hepatomegaly and splenomegaly occurred in one case while a decrease in splenomegaly alone occurred in another case receiving ACTH

Of the cortisone treated group the only noteworthy effect was amelioration of anorexia in one patient On the other hand an in crease in liver size of approximately 2 cm was observed in another case following cortisone

Slight reduction of total serum bilirubin was observed in 5 cases in both the groups The significance of this reduction is questionable however since in none of the cases was total serum bilirubin elevated above 2 mgs /100 ml initially Of the remainder of the observations perhaps the most consistent was an increase in serum cholesterol an immediate increase in urinary coproporphyrin excretion of from 1 to 17% of the pretreatment values and an immediate decrease in bromo

DR ROBERT KARK We have noted high levels of urinary adrenal cortical hormones and very low levels of 17 ketosteroids in urine from patients who were very ill with cirrhosis of the liver (Table V) This adrenal disassociation disturbed us for we find in our patients at least no evidence of hypertension increase in body weight and change in structure of Cushing s syndrome type The only Cushing like lesions we find are occasionally acne with of course marked sodium retention

Our attempts to show in our laboratory that the increases in cortin were due specifically to salt retaining hormone have been most disappointing We have been completely dissatisfied with the methods that have been available for clinical measurement of 11 desoxy

Table V

24 Hour 17 Ketosteroids and Cortin Excretion in the Urine from 6 Patients with Cirrhosis of Liver

No	*Patient*	*Age*	*Sex*	*Blood Pressure*	*Urinary* Cortin mg per 24 hours*	*Urinary 17 Ketosteroids mg per 24 hours*	*Eosinophiles per MM³*
1	L F	58	M	116/68	4 5	1 6	100
2	T W	58	M	118/62	3 8	0 75	140
3	K K	54	M	112/72	3 3	1 8	343
4	A M	32	M	98/56	4 5	1 2	66
5	P W	52	M	108/62	5 7	2 2	121
6	B F	60	M	128/70	3 8	1 4	143

Normal Range 0 5–2 0 mg /24 hours

corticosterone We tried to test our hypothesis that ascites edema and sodium retention were the result of excess adrenal activity by salt loading tests

In six patients with cirrhosis of the liver on a low sodium diet to whom we gave salt two showed increases of cortin at the time of salt loading but the other four showed no increases

We then did rather prolonged metabolic studies on four patients with cirrhosis of the liver First using ACTH therapy and salt loading as a test method The only striking findings were differences between sodium chloride loading when there is a gain in weight with no sharp water diuresis following cessation of salt Whereas with ACTH and salt there is a sharp gain in weight and then a rapid rebound with diuresis Other than this there is no clear evidence to help us in our concept that by stimulating the adrenal gland we might depress the salt retaining hormones with a reciprocal increase in other cortical hormones

The other patients with hepatic cirrhosis were not benefited in any substantial way by ACTH In 1 patient with a pre treatment thrombocytopenia the platelets returned to normal during therapy but the thrombocytopenia returned promptly after stopping ACTH There was no significant change in the liver function tests in any of the cases studied on the other hand there was a decrease in the serum gamma globulin concentration of the order of 0 4 gm per 100 cc serum in all but 1 patient The total globulin decreased a correspond ing amount

Six of the cirrhotic patients were unusually fatigued beginning about the seventh day of therapy and 1 patient became psychotic on

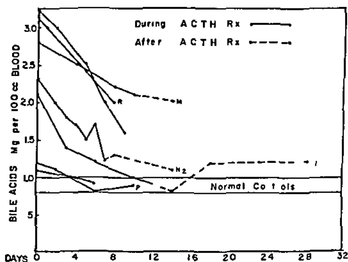


FIG 8

the sixth day but recovered in 4 days Ascites developed in 2 patients but not in the others One patient was comatose and moribund when therapy was started She lived for six days thereafter but it is doubt ful that ACTH altered the course in any way

In conclusion it is our impression that no sustained or permanent benefit was derived in any patient On the other hand it is doubtful that ACTH was harmful in any of the cases The striking correlation between pruritus and the bile acid level may be significant and sug gestive of the importance of bile acids in the production of pruritus in jaundice patients

DR SEYMOUR J GRAY (Peter Bent Brigham Hospital and Harvard Medical School Boston) We have noticed a decrease in alkaline phosphotase after ACTH administration in some patients with cir rhosis and we wondered if you have made a similar observation

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The Role of the Adrenal Cortex in the Regulation of the Plasma Iron Level and the Functional State of the Reticulo Endothelial System*

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It was reported from this laboratory in 1946[1] that following the intramuscular injection of turpentine into dogs marked hypo ferremia appears within 24 hours and reaches a maximum in 24 to 48 hours It was subsequently demonstrated that the intravenous in jection of colloidal thorium dioxide in dogs is associated with pro nounced hyperferremia and that following the injection of thorium the hypoferremia producing effect of turpentine does not develop[2] Since both iron and colloidal thorium are in large part removed from the circulation by the macrophagic tissues one possible explanation of the above observations is that the injection of turpentine in some way enhances the functional state of the reticulo endothelial system so that iron is rapidly removed from the plasma while the repeated administration of colloidal thorium results in a temporary functional depression of the reticulo endothelial system at least in so far as iron metabolism is concerned[3]

The purpose of the experiments reported here is to elucidate fur ther the mechanism by which hypoferremia is produced in the dog as well as in the rat For these studies approximately 200 mongrel dogs and 500 male Sprague Dawley rats were used The details of the experiments will be published elsewhere[4,5]

In order to determine whether stresses other than turpentine are accompanied by hypoferremia the effects of a variety of agents and procedures including histamine epinephrine formaldehyde ana phylactic shock fracture of the femur and the stress of taking blood samples were studied The results are presented in Table I Hista mine epinephrine formaldehyde and anaphylactic shock all produce

* This study was supported by grants from the United States Public Health Service and the Upjohn Company

We then studied the same patients on cortisone for a period of 18 days hoping we would depress the 11 desoxy compounds and cause a diuresis The patients went into negative sodium balance at the end of cortisone therapy This became more marked when cortisone was withdrawn and at the time the adrenal gland is depressed which may be a cortisone depot effect In any case there is no clear evidence in our data to suggest that sodium retention in cirrhosis is due to 11 desoxycorticosterone excess We still hope to show that ascites in cirrhosis is related to overproduction of 11 desoxycorticosterone

As far as the clinical side is concerned none of these patients showed any improvement In fact three of them became much more ill during the time they were on cortisone therapy Their livers en larged rather rapidly One developed dementia with a very marked depression and two of them had attacks of high fever during the course of the injections

DR MARCEL ROCHE (Cascarita Hospital Caracas Venezuela) With the collaboration of Dr Otto Lima Gomez at the Hospital Vargas in Caracas Venezuela we have given ACTH 80 mg per day to a case of Schistosomiasis mansoni with a Banti like syndrome with cir rhosis of the liver and a very large spleen

Clinically there was practically no effect and no change in the size of the spleen could be observed On the laboratory side there was a definite tendency for the globulin pattern to revert to normal The original eosinophil level was low 59 per cu mm of blood to be ex act On the other hand leukopenia was also present and I am won dering whether this is not an important factor in the production of eosinopenia rather than the increased gluco corticoid production as Dr Bongiovanni has postulated?

DR ALFRED M BONGIOVANNI (Philadelphia Pa) In relation to Dr Roche s remarks I am not sure why the initial eosinophil count is low only in some In all cases of cirrhosis we have found somewhat elevated corticoids As regards the patients with Cushing s syndrome and cirrhosis we don t know which came first since the patients were referred to us because of liver diseases On the basis of history from the mothers of some of these patients and the patients themselves their first indication of illness was jaundice Sometimes they did re member that they had striae and had been shaving for some time be fore the jaundice but that the liver disease did not exist first we can not be certain

Concerning low sodium that is our treatment for cirrhosis and the convalescent patient I showed you with the rise in the glucuroni dated ketosteroids had been treated with a low sodium diet

crease in the plasma iron was not significantly different from the changes observed in intact animals injected with turpentine

Additional evidence of an association between the adrenal cortex and the level of plasma iron was afforded by experiments on the rate of disappearance from the plasma of intravenously administered saccharated oxide of iron This substance was removed most rapidly from the plasma of intact dogs given ACTH and most slowly from the plasma of adrenalectomized dogs the rate of disappearance in intact untreated animals falling between these two The rate of disappearance in dogs given thorium dioxide was approximately equal to that in adrenalectomized dogs

It is noteworthy that a significant degree of eosinopenia and of lymphocytopenia accompanied the hypoferremia which followed the administration of turpentine epinephrine and ACTH (Table II)

Table II

MAXIMAL CHANGES IN THE LEVEL OF EOSINOPHILS AND LYMPHOCYTES FOLLOWING THE INJECTION OF A VARIETY OF AGENTS IN INTACT AND ADRENALECTOMIZED DOGS

Substance Injected	*Dose*	*Adrenal Glands*	*No of Dogs*	*Eosinophils per cent Change*	*Lymphocytes per cent Change*
Turpentine	0 5 ml	Intact	10	−81	−42
		Removed	4	−38	+15
Epinephrine	2 mg	Intact	5	−80	−62
		Removed	6	+19	+33
ACTH	25 mg	Intact	10	−84	−30
		Removed	6	+50	+21
Saline	5 ml	Intact	10	−30	−21
		Removed	6	+30	+36

Following the injection of saline a significant fall in the eosinophils and lymphocytes only took place in those dogs which developed hypoferremia In adrenalectomized animals turpentine was the only substance which produced a significant decrease in the number of circulating eosinophils However this decrease was less marked than in intact animals given turpentine In the absence of the adrenal cortex epinephrine produced hypoferremia which was significantly less pronounced than that observed in the intact dog This was furthermore not associated with a significant decrease in eosinophils Here then is further evidence that the full hypoferremia producing effect of epinephrine can only be elicited in the presence of the adrenal cortex It is also clear however that epinephrine acts in part through another mechanism

a marked lowering of the plasma iron Maximal hypoferremia appeared 8 hours after the injection Fracture of a femur also was followed by hypoferremia Here however just as occurs following the injection of turpentine maximal hypoferremia appeared 24 hours later The injection of saline and the stress of taking blood specimens resulted in a modest hypoferremia in 8 hours

In view of these findings the effects of the administration of adrenocorticotrophic hormone and of lipo adrenal extract were studied

Table I

THE HYPOFERREMIA PRODUCING EFFECT OF A VARIETY OF AGENTS IN INTACT AND IN ADRENALECTOMIZED DOGS

Substances Injected or Experimental Procedure	*Dose*	*Adrenal Glands*	*No of Dogs*	*Maximal Mean Decrease in Plasma Iron per cent*	*Time of Maximal Decrease in Plasma Iron hours*
Turpentine	1 ml	Intact	7	82	24
Histamine	25 mg	Intact	7	61	8
Epinephrine	2 mg	Intact	18	53	8
	2 mg	Removed	9	26	8
Saline	5 ml	Intact	8	24	8
	5 ml	Removed	11	5	8
ACTH	25 mg	Intact	7	47	8
	25 mg	Removed	6	10	8
Lipo-adrenal cortical extract	30 ml	Intact	5	50	8
Formaldehyde	5 ml	Intact	1	46	8
Fracture		Intact	2	60	24
Anaphylactic shock*		Intact	1	80	8

* Anaphylactic shock was produced by the intravenous injection of 10 ml. of horse serum into a dog previously sensitized with horse serum

The results are presented in Table I The injection of both ACTH and of cortical extract was followed by a decrease in plasma iron which was maximal in 8 hours Similar results were observed after the injection of 30 ml of lipo adrenal extract

In adrenalectomized animals neither ACTH administration nor the taking of blood specimens was associated with hypoferremia The hypoferremia producing effect of epinephrine was significantly less in adrenalectomized dogs than in intact animals but epinephrine still produced a significant fall in plasma iron in the absence of the adrenal cortex Adrenalectomized dogs given histamine or turpentine failed to survive 24 hours but five adrenalectomized dogs survived 8 hours after the injection of turpentine At this time the per cent de

tration of 0 5 mg/rat/day of desoxycorticosterone acetate did not prevent the development of the post adrenalectomy hypoferremia

One possible explanation of the acute hypoferremia observed in our experiments is that the cortical hormones stimulate the reticulo endothelial system to take up iron from the plasma In support of this explanation is the observation of Gordon and Katsh[8] that in

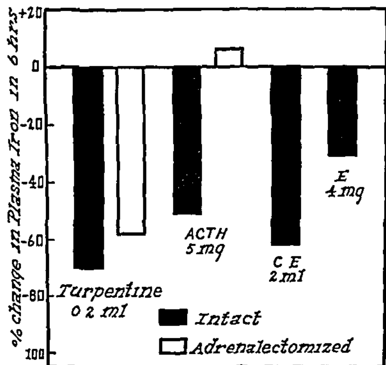


Fig 2 The level of plasma iron in normal sham operated and adrenal ectomized rats and in adrenalectomized rats supported with lipo-adrenal cortical extract cortisone (Compound E) and desoxycorticosterone acetate

rats adrenalectomy results in a significant decrease in the uptake of thorium by the spleen while the administration of whole adrenal cortical extract significantly increases the accumulation of thorium in the spleens of adrenalectomized animals

This explanation is admittedly entirely speculative Contradicting it is the fact that in the rat we have been unable to produce hyperferremia by the injection of colloidal thorium dioxide Furthermore studies to date in human subjects with ACTH and cortisone have not confirmed the findings in rats and dogs

It is possible that ACTH and cortisone produce hypoferremia

In the rat as in the dog the administration of turpentine (0 2 ml) histamine (150 mg) ACTH (5–10 mg) lipo cortical extract (2 0 ml) or cortisone (4–8 mg) was followed by hypoferremia (Figure 1) Epinephrine however failed to produce hypoferremia in this species Adrenalectomy completely abolished the hypoferremia producing effect of ACTH but not that of histamine turpentine or cortical extract

Quite unexpectedly however it was found that in the rats adrenalectomy *per se* resulted in a significant decrease in the plasma

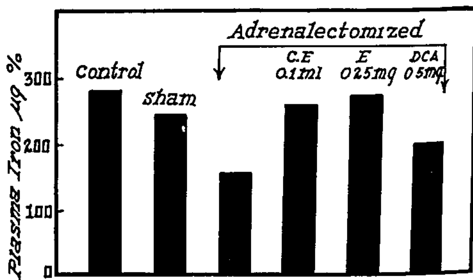


FIG 1 The hypoferremia producing effect of turpentine and ACTH in intact and in adrenalectomized rats and of lipo cortical extract and cortisone (Compound E) in intact rats

iron level Sham operations had little or no effect on the plasma iron level (Figure 2) In the dogs it was noted that the initial plasma iron levels were all lower in the adrenalectomized groups than in the corresponding intact groups but in only one adrenalectomized group was the initial iron level sufficiently lower than in the control group to be statistically significant The dogs in this group had been maintained largely on desoxycorticosterone acetate in contrast to the other adrenalectomized dogs which had been supported with small doses of adrenal cortical extract

Because of these observations rats were adrenalectomized and maintained on small supportive doses (0 25 mg/rat/day) of cortisone for three days Under these conditions the post adrenalectomy hypoferremia did not develop (Figure 2) On the other hand the adminis

DISCUSSION

DR EDWARD FISCHEL (Presbyterıan Hospıtal and Columbıa Unıversıty College of Physıcıans and Surgeons New York Cıty) In a patıent with anemıa associated with rheumatıc fever the ıron bındıng pro teın was low When rheumatıc actıvıty was controlled by ACTH the ıron bındıng proteın rose and the anemıa was subsequently cor rected In thıs case at least the admınıstratıon of ACTH caused a change ın the dırectıon opposıte to that whıch occurred ın Dr Cart wrıght s dogs

Whıle ACTH may cause a drop ın ıron bındıng proteın of the normal ındıvıdual ıt appears that ıt wıll result ın a rıse of thıs sub stance to normal by suppressıng the ınflammatory process prevıously responsıble for a low ıron bındıng proteın level

The studıes presented by Dr Cartwrıght and hıs coworkers are of extreme ımportance However I would take ıssue wıth the hypoth esıs that the retıculo endothelıal system ıs necessarıly concerned with the mechanısm suggested It ıs well known that ACTH results ın atrophy and destructıon of lymphoıd tıssue ıncludıng the retıculo endothelıal cells It may be antıcıpated therefore that thorıum mıght not be stored as readıly durıng hormone admınıstratıon

DR IRVING SCHULMAN (New York Hospıtal and Cornell Unıversıty Medıcal College New York Cıty) We have studıed the serum ıron and ıron bındıng capacıty ın four chıldren wıth Medıterranean Ane mıa treated wıth ACTH The serum ıron ın these chıldren has been found to average 269 gamma percent as compared to 155 gamma per cent found ın normal chıldren The latent ıron bındıng capacıty ın severe Medıterranean Anemıa ıs zero as compared to 200 gamma per cent ın the normal

Upon ACTH admınıstratıon each of the four chıldren demon strated a decrease ın serum ıron level at five to nıne days from the onset of therapy The decrease averaged 30% of the orıgınal values However durıng the second week the serum ırons rose agaın to pre treatment levels It ıs ımportant to poınt out that constant hemolysıs probably occurs and ıs an ımportant factor ınfluencıng the serum ıron levels

Possıbly of greater sıgnıfıcance was the observatıon of a return of a latent ıron bındıng capacıty ın two cases These values were 100 and 150 gamma percent from a prevıous zero and occurred on the nınth and fourth day respectıvely The return of a latent capacıty was not dependent upon a lowerıng of the serum ıron for the total capacıty

through an entirely different mechanism than does turpentine In deed it has been shown in the present study that epinephrine and turpentine can produce some degree of hypoferremia even in adrenal ectomized dogs Whether or not this is by direct stimulation of the reticulo endothelial system or through some intermediary channel other than the pituitary adrenal axis are possibilities which are re ceiving further study

The apparent paradox that in the rat both adrenalectomy and the administration of a very large amount of cortisone produce a decrease in the plasma iron level may mean that the adrenal hor mones exert a regulatory effect on the level of iron circulating in the plasma Further studies are necessary The present investigations merely indicate that there is a relationship between this endocrine gland and iron metabolism

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The Effect of Adrenocorticotrophic Hormone (ACTH) and Cortisone on the Total Coproporphyrin Excretion in Humans*

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In an investigation of the effect of isotopic (I^{131}) radiation and x radiation on the coproporphyrin excretion in humans we have observed an increase in the excretion of coproporphyrin in the urine and feces in six patients who had been subjected to therapeutic amounts of ionizing irradiation[1] A seventh patient who received x radiation to the adrenal regions has been the only patient in whom we did not observe an increase in coproporphyrin excretion Figure 1 graphically represents the urinary excretion of coproporphyrin in one of the studies done in the course of this investigation It is included here for comparison with the observations to be reported in this paper During the course of this study Lawrence[2] reported that the 17 ketosteroid levels in the urine of dogs exposed to minimal lethal doses of x radiation increased in all irradiated animals five to twelve days after exposure Later Edelmann[3] protected the adrenals of male rats from x rays by means of lead shields and noted a significant increase in survival incidence in the animals so protected Conversely he observed that x radiation shortens the survival time of adrenalectomized rats from an average of eight days to four days Cronkite and Chapman reported similar observations in a study on mice

With these various observations in mind we have been following the coproporphyrin excretion in a group of patients receiving ACTH It is the purpose of this paper to present the results of this study and to compare them with the data obtained from patients treated with ionizing irradiation

* The authors are indebted to Mrs James Crawford and Mr Milton Greenberg for their aid and assistance

was greater than the pre treatment values These observations may indicate an effect of ACTH on the iron binding protein

DR STUART FINCH (Peter Bent Brigham Hospital and Harvard Medical School Boston) I should like to ask Dr Cartwright if iron binding capacity was altered by the administration of ACTH?

DR G E CARTWRIGHT I would like to warn against the direct application of these studies in dogs to human subjects To date we have not observed the same results in human subjects that we have in the animals In the human subjects more variables are present than in the animals

As suggested by our studies with rats I am not surprised that in an infectious process plasma iron rose as the infectious process subsided The rat studies would suggest that the adrenal cortex plays a regulatory role rather than one that is either causing hypo or hyper ferremia

We are delighted to have someone criticize the hypothesis We would indeed be surprised if it stood the test of time We have not measured the iron binding capacity in the dogs and rats of this study We can say that dogs given turpentine have a decrease in the total iron binding capacity but this decrease does not seem to be great enough to account for the hypoferremia

tions There was no change in the diets received by these patients during the period of study

The procedures for the extraction of coproporphyrin from the urine and feces are based on the methods of Schwartz and Watson[5] and on those of Dobriner[6] They have been presented in detail in a previous report[1]

CLINICAL COURSE AND LABORATORY FINDINGS

Case I

The first study was done on a 59 year old male (F K M H #93994) who was admitted to Memorial Hospital with a provisional diagnosis of gastric carcinoma No evidence of malignancy was found Beginning on August 3 1949 he was given 100 mg of Armour s adrenocorticotrophic hormone (ACTH) daily for ten days

The data obtained in this study are summarized in Figure 2

Coproporphyrin Excretion Only the urinary coproporphyrin excre

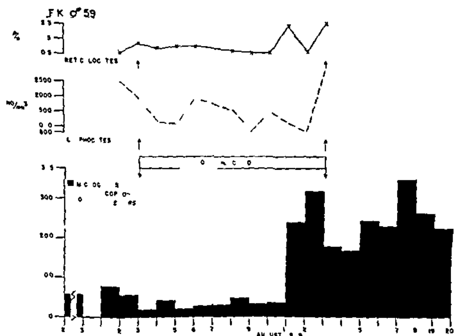


Fig 2 Urinary coproporphyrin excretion and peripheral blood studies on F K M H #93994 (Case 1) The solid black blocks represent the urinary coproporphyrin content per 24 hours The period of treatment with ACTH is delineated by arrows

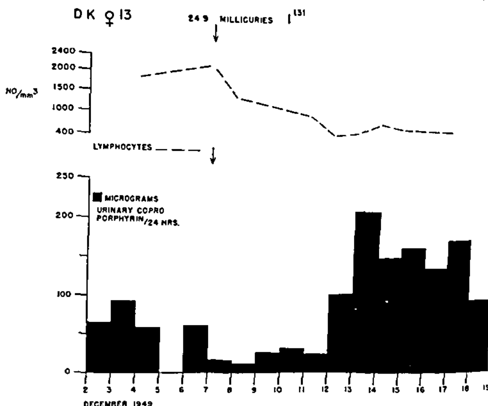


Fig 1 Urinary coproporphyrin excretion and peripheral blood studies on D K M H #89431 a 13 year old girl with carcinoma of the thyroid with metastases to the lungs She was treated with 24 9 millicuries of I^{131} as indicated by the diagram The solid black blocks represent the urinary coproporphyrin content per 24 hours

MATERIALS AND METHODS

The five patients included in this study have all been under treatment at the Memorial Hospital Three patients received ACTH alone One of them was under observation for possible carcinoma of the stomach A second received ACTH in the treatment of osteitis pubis The third had Graves disease A patient with idiopathic thrombocytopenic purpura received ACTH during the first part of the study and was later treated with cortisone (Compound E) The treatment period in the fifth patient was divided into three stages In the first and last he received ACTH alone During the middle period he received cortisone in addition to ACTH He was under treatment for lymphosarcoma No medication except as indicated in the clinical report was administered during the course of these observa

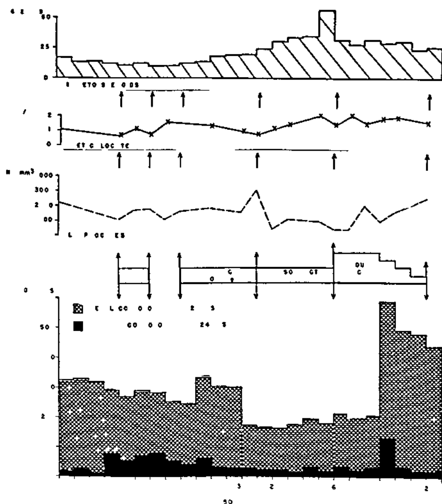


FIG 3 Total coproporphyrin excretion peripheral blood studies and 17 ketosteroid excretion on S P M H #100551 (Case 2) The solid black blocks represent the urinary coproporphyrin excretion per 24 hours the stippled blocks represent the fecal content per 24 hours The arrows delineate the period of treatment and the bars represent dosage

was an abrupt rise in both fecal and urinary coproporphyrin excretion to well above pretreatment levels

Hematologic Findings There was a gradual increase in the numbers of circulating reticulocytes during the period of therapy On the ninth day of continuous treatment with ACTH they were slightly

tion was followed in this case The pretreatment coproporphyrin levels were within normal limits[7] There was a decrease in coproporphyrin excretion on the first day of ACTH administration It continued for eight days On the following day the level increased to three times that observed during the control period and continued at well above pretreatment values throughout the remaining nine days of the study

Hematologic Findings A decrease in the number of circulating lymphocytes occurred on the second day and continued until the final day of treatment with ACTH Concomitantly the white count increased and remained high throughout the period of observation Eosinophils disappeared from the peripheral circulation on the second day There was a gradual decrease in the hemoglobin level and hematocrit during the period of study A reticulocytosis occurred on the same day that the coproporphyrin excretion was observed to increase There was no change noted in the platelet count

Case II

A sixty one year old male (S P M H #100551) was admitted to Memorial Hospital on May 18 1950 with a diagnosis of osteitis pubis After a three day control period 100 mg of ACTH was administered daily May 23rd to May 25th inclusive Following this sterile saline injections every 6 hours were administered for two days On May 27 1950 100 mg of ACTH per day was again resumed and continued until June 6 1950 when 200 mg per day was given ACTH was gradually decreased to zero beginning June 9 and ending June 12 1950 The patient received codeine and aspirin on May 26 1950 and again on June 3 1950 Aureomycin 2 0 gm daily was given for a urinary tract infection from June 9 1950 to June 13 1950 the day on which he was discharged from the hospital

The coproporphyrin excretion hematologic data and results of 17 ketosteroid assay are summarized in Figure 3

Coproporphyrin Excretion During the control period of observation the total coproporphyrin excretion was noted to be within the normal limits The greater fraction was contained in the fecal component There was no significant change in either the urinary or the fecal coproporphyrin excretion until the eighth day of the treatment period the fourth day of continuous treatment with ACTH At that time the fecal coproporphyrin levels decreased to about half those observed during the control period They remained at this lower level during the next nine days On the twelfth day of continuous treatment with ACTH three days after the dose had been doubled there

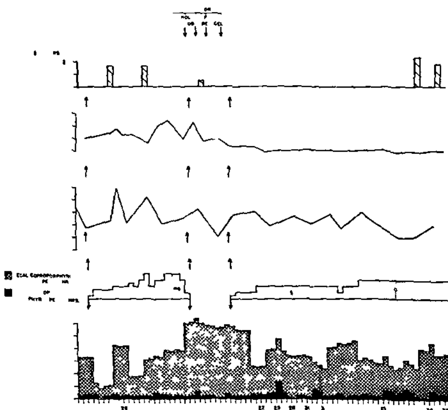


FIG 4 Total coproporphyrin excretion peripheral blood studies and 17 ketosteroid excretion on B A M H #101084 (Case 3) The solid black blocks represent the urinary coproporphyrin excretion per 24 hours the stippled blocks represent the fecal content per 24 hours The arrows delineate the period of treatment and the bars represent dosage

tion continued for a total of thirteen days remaining high until four days after the last red blood cell transfusion There was no rise in the coproporphyrin excretion during the period of therapy with cortisone Indeed it appears that cortisone caused a fall from the levels observed at the end of treatment with ACTH However this decrease was not below the pretreatment levels

Hematologic Findings The reticulocytes which were markedly increased at the onset of the study fluctuated well above normal levels until four days after hemoglobin had been restored to normal by transfusions They remained within normal limits during the period

more than 2% at which level they remained throughout the final days of the study There was a concomitant gradual rise in hemo globin content There was no significant change in the total number of circulating lymphocytes though a tendency towards a decrease in their numbers was noted on the fourth day of continuous ACTH The white cell count gradually increased to a maximum of 17 000 on the twelfth day of continuous treatment It is interesting to note that the eosinophils did not disappear from the peripheral blood until the fifth day of continuous ACTH

17 Ketosteroid Excretion As was noted in the eosinophil count the 17 ketosteroid excretion did not change until the fifth day of continu ous therapy with ACTH At that time a step—wide increase in the urinary steroids—occurred It reached a peak on the ninth day fol lowing which levels above those noted in the control period were maintained throughout the remainder of the study

Case III

The third study was done on a forty three year old housewife (B A M H #101 084) who was admitted to Memorial Hospital on June 25 1950 with a diagnosis of thrombocytopenic purpura of one year s known duration On July 17 1950 a control period was begun and on July 19 1950 the first dose of ACTH was given As indicated in Figure 4 ACTH was administered daily in varying dosage through August 7 1950 From August 7 until August 16 1950 the patient re ceived no further hormonal treatment Two transfusions of 500 cc of whole blood and two transfusions of 500 cc of packed red blood cells were given during this interim period On August 16 1950 and continuing throughout the remainder of the study she received cor tisone in the doses indicated in Figure 4

The coproporphyrin excretion hematologic data and 17 ketoster oid excretion are summarized in Figure 4

Coproporphyrin Excretion During the brief control period which preceded ACTH administration the total coproporphyrin excretion was within normal limits It decreased to below normal on the second day of treatment remained low for four days then rose to slightly above control levels for three days A second decrease in total copro porphyrin excretion was observed to occur on the eighth day of treat ment It continued for three days following which a gradual increase to above pretreatment levels was noted Two days before treatment with ACTH was discontinued there was an abrupt increase in the fecal coproporphyrin excretion with no change in the urinary copro-porphyrin excretion Above normal levels of coproporphyrin excre

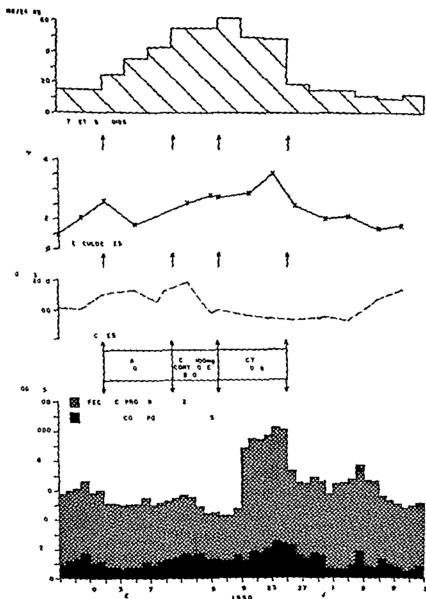


FIG 5 Total coproporphyrin excretion peripheral blood studies and 17 ketosteroid excretion on M J M H #100600 (Case 1) The solid black blocks represent the urinary coproporphyrin excretion per 24 hours the stippled blocks represent the fecal content per 24 hours The arrows delineate the period of treatment and the bars represent dosage

of cortisone during which same period the hemoglobin maintained itself at normal levels No significant change in the total number of circulating lymphocytes was noted nor did the total white cell count vary throughout the period of observation The total number of circulating eosinophils increased to two times the pretreatment level on the third day of treatment with ACTH They remained above the levels observed during the control period until the sixteenth day of treatment with ACTH and did not disappear from the circulating blood until the sixth day of treatment with cortisone The platelets were first noted in the peripheral blood on the ninth day of cortisone therapy

17 Ketosteroid Excretion Only five determinations of the urinary excretion of 17 ketosteroids were done during the period of study All were within normal limits

Case IV

The fourth patient studied was a 45 year old male (M J M H #100600) who was admitted to Memorial Hospital on May 23 1950 with a diagnosis of lymphosarcoma of three and one half years known duration On May 25 1950 a metabolic balance study* was begun and from June 1 to June 25th inclusive the patient received 100 mg of ACTH daily Beginning on June 10 1950 and continuing for six days he received 200 mg of cortisone daily in addition to ACTH He received Nembutal 0 1 gms daily throughout the period of study

The coproporphyrin excretion hematologic data and results of 17 ketosteroid assays are summarized in Figure 5

Coproporphyrin Excretion During the pretreatment period of study the total coproporphyrin excretion was almost twice the normal levels During the first twenty four hours of treatment with ACTH there was a slight decrease in the fecal and urinary excretion of coproporphyrin This decrease in fecal coproporphyrin excretion continued until the 4th day of treatment with cortisone when a second decrease in total coproporphyrin excretion was observed On the nineteenth day of treatment 3 days after treatment with ACTH alone was resumed there was a sharp rise in the fecal coproporphyrin excretion to well above control levels It continued until the twenty fifth day of treatment following which it gradually returned to the levels noted during the pretreatment phase on the thirty sixth day of the study eleven days after the administration of ACTH was discontinued On the sixth day of treatment the urinary coproporphyrin excretion be

* This patient is one of several patients who have been followed in a metabolic study which is being conducted by Drs Olof H Pearson and Leonard P Eliel in the Department of Clinical Investigation at the Sloan Kettering Institute

LG ♀ 24

FIG 6 Total coproporphyrin excretion peripheral blood studies and 17 ketosteroid excretion on L G M H #97546 (Case 5) The solid black blocks represent the urinary coproporphyrin excretion per 24 hours the stippled blocks represent the fecal content per 24 hours The arrows delineate the period of treatment and the bars represent dosage

during the period of therapy An increase to 3% of the total circulating red cells occurred on the third day of the post treatment period It continued upward to a peak of 4% reticulocytes on the fifth

gan to rise It continued at or above the levels noted during the control period until five days after ACTH had been discontinued

Hematologic Findings A gradual increase in the total number of circulating reticulocytes was noted during the period of therapy A 3% reticulocytosis was noted on the 11th day of ACTH It gradually increased to a peak of 4% on the twenty third day of treatment There was a gradual decrease in hemoglobin throughout the entire period of observation An initial increase in the lymphocyte count was followed by an abrupt decrease to below control levels during the period of combined ACTH and cortisone therapy No significant change was noted in the total white cell count nor in the platelet count The eosinophil count dropped to zero on the fifth day of treatment

17 Ketosteroid Excretion The 17 ketosteroid assay showed a step wise increase which began on the first day of therapy and reached a peak on the sixteenth day of treatment with ACTH one day after combined treatment with ACTH and cortisone was discontinued During the post treatment phase the 17 ketosteroids abruptly fell to below control levels

Case V

The fifth patient a 24 year old married negress (L G M H #97546) was admitted to Memorial Hospital on October 17 1949 with a diagnosis of Graves disease On October 18 1949 treatment with phenobarbital 0 6 gm four times daily was started On October 24 1949, 50 mg of ACTH daily was begun and on October 26 1949 phenobarbital was discontinued The daily dose of ACTH was increased to 100 mg on November 2 1949 and to 200 mg on November 15 1949 Administration of ACTH was discontinued on November 18 1949 and the following day treatment with potassium iodide 0 6 gm was started

The coproporphyrin excretion hematologic findings and 17 keto steroid excretion are summarized in Figure 6

Coproporphyrin Excretion The total coproporphyrin excretion during the control period was at the upper limit of normal At the onset of treatment and continuing throughout the period of treatment with ACTH the total coproporphyrin gradually decreased The average excretion during the control period was 287 micrograms per 24 hours and during the treatment period 232 micrograms per 24 hours During the post treatment period the average excretion was 269 micro grams per 24 hours *A relative increase in the urinary excretion of* coproporphyrin occurred during the period of teatment and persisted throughout the study

Hematologic findings The reticulocytes were within normal limits

The theoretical mechanisms by which the increased coproporphyrin excretion may be brought about have been the subject of discussion in a previous report[1] and will not be included here

SUMMARY AND CONCLUSIONS

1 The excretion of coproporphyrin in the urine and feces has been studied in five patients receiving ACTH Two of these patients received cortisone in addition to ACTH

2 A sixth patient who received radioactive iodine (I^{131}) in the treatment of carcinoma of the thyroid is included for comparison

3 The observations of the coproporphyrin excretion have been correlated with studies of the peripheral blood

4 The four patients in whom an increase in coproporphyrin excretion was noted exhibited an increase in reticulocytes and a decrease in circulating lymphocytes and eosinophils These changes were not noted in the patient who did not reveal an increased excretion of coproporphyrin

5 The changes observed in patients treated with ACTH are similar to those observed in patients treated with ionizing irradiation

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DISCUSSION

DR ROBERT KARK You did not differentiate total coproporphyrin into isomers one or three?

DR HENRY J KOCH JR We were measuring total coproporphyrin A study is in process now to measure the isomers

post treatment day There was no change in the hemoglobin concentration until nine days after ACTH was stopped At that time a hemoglobin of 16 9 gm % was recorded The total number of circulating lymphocytes and the total white cell count were not significantly changed during the period of ACTH administration Eosinophils were present in the peripheral blood throughout the course of the study

17 Ketosteroid Excretion There was an immediate decrease in 17 ketosteroid excretion when ACTH was discontinued

GENERAL DISCUSSION

The coproporphyrin excretion was within normal limits during the control period in four of the patients studied A fifth patient (Case 4) with lymphosarcoma was observed to have an increase in the total coproporphyrin excretion per 24 hours during the pretreatment period It was approximately twice the reported normal levels[7] and was noted in both the fecal and urinary fractions

An increase in total coproporphyrin excretion was observed in four of the five patients treated with ACTH, The fifth patient (Case 5) exhibited a shift in coproporphyrin from fecal to urinary excretion but the total excretion was decreased during the period of treatment with ACTH In addition no change in reticulocytes lymphocytes or eosinophils was noted during the period of therapy

No definite time relationship could be found between the onset of ACTH administration and the increase in coproporphyrin excretion In general the period of increased coproporphyrin excretion coincided with the period of reticulocytosis and occurred eight to ten days after the initial decrease in circulating lymphocytes and eosinophils In all four patients who exhibited an increased coproporphyrin excretion an initial decrease in total coproporphyrin was observed Only two of the patients were observed for an extended period after the administration of ACTH was discontinued In these the total coproporphyrin excretion remained above control levels until twelve and thirteen days after cessation of treatment

In the two patients who received cortisone as well as ACTH no increase in the coproporphyrin excretion was noted during the period of cortisone administration Contrariwise the patient (Case 4) who received ACTH and cortisone simultaneously revealed a tendency toward a decreased total coproporphyrin excretion during the period of combined therapy followed by an abrupt increase after cessation of cortisone The other (Case 3) showed a marked fall in coproporphyrin excretion on the fourth day of cortisone administration However such observations can only be considered coincidental until further studies can be carried out

CASE REPORTS

Case 1

Mrs F G age 59 was admitted to the R V H January 7 1950 The history of her present illness was that of moderate pallor for many months dyspnoea after slight exertion tiredness and severe pallor for one month nausea and vomiting after meals tingling and numbness of the toes for 10 days and slight vaginal bleeding 2 days During the several months before admission she had lost some 16 pounds Personal and family histories were irrelevant Physical ex amination showed dyspnoea on slight exertion marked pallor sub icteric sclerae small haemorrhages in the left fundus oculi The liver was palpable 3 fingerbreadths below right costal margin The neuro logical findings were absent vibration sense over the left ankle and diminished over the right hyperactive knee jerks and ankle jerks bilateral Babinski s sign At times she was disoriented confused and hallucinated speech was thick

General laboratory findings were Gastric analysis Jan 9 before ACTH and Jan 26 after ACTH no free HCl after histamine Uri nalysis was repeatedly negative Direct bilirubin 0 8 mgs % total 2 0 mgs % on Jan 17 Barium meal Feb 6 normal except for a seg mented pattern of barium in the proximal jejunum suggesting motor dysfunction Urinary 17 ketosteroids Jan 10 4 3 mgs in 24 hours Jan 13 29 0 mgs in 24 hours Several stool specimens were negative for fresh and occult blood

Initial hematological findings on Jan 7 RBC 1 350 000 Hgb 5 2 gms MCV 122 cu microns MCH 38 γγ MCHC 31 % hemato crit 16 5% reticulocytes 4–5% Leucocyte count was 7 600 with 76% neutrophiles 1% eosinophiles 21% lymphocytes and 2% mono cytes Platelet count was 140 000 The serum was slightly icteric Sternal marrow aspiration on Jan 9 showed megaloblastic hyperpla sia and many macrogranulocytes

From Jan 7th to the 11th there was mental deterioration in creasing dyspnoea and tachycardia accompanying the grave anemia The red cell count fell to 970 000 Consequently she was given a transfusion of 500 cc of packed red cells which brought the red count to 2 620 000 and hemoglobin to 7 6 gms

ACTH was given in doses of 25 mg every 6 hours by intramus cular injection beginning Jan 11 A total of 875 mgs was given up to Jan 20 During this period mental symptoms became more marked and there was no improvement in the neurological signs Large pur puric areas developed over the face trunk and lower extremities and there was increasing edema

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The Effect of Adrenocorticotrophic Hormone on the Blood and Bone Marrow of Pernicious Anemia

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QUEEN MARY VETERANS HOSPITAL AND MCGILL UNIVERSITY MONTREAL

In January and February of 1950 two patients suffering from pernicious anemia both previously untreated were administered single short courses of ACTH and subsequently Vitamin B 12 The case histories of these two patients and the hematological effects of ACTH upon them are reported in detail

Since the complete trial of ACTH upon these two patients Wintrobe[1] has reported that ACTH produced no clinical or hematological improvement in patients with pernicious anemia In May of 1950 Thorn and others[2] reported Gardener s case of pernicious anemia who was given 480 mg of ACTH over 8 days A reticulocytosis ensued which reached a peak of 7 5% on the ninth day although there was no increase in the total count Bone marrow aspiration showed a temporary increase in the maturation level of nucleated red cells ACTH produced a more profound effect in our two patients and in patient No 2 a more lasting effect

Hematological studies included red blood cell hematocrit hemoglobin reticulocyte leukocyte blood platelet and differential estimations performed at frequent intervals Serial studies of the bone marrow were obtained by puncture Total nucleated cell counts were obtained and differential smears were made by the cover slip method without the aid of anti coagulant and were stained with Jenner Giemsa *Each differential count was continued until a total of 400* nucleated red cells were counted In these counts the classification of Jones[3] Leitner[4] Wintrobe[5] etc was used with slight modification Some authors especially Dacie and White[6] have noted the presence of nucleated red cells which are intermediate between the megaloblastic and normoblastic series Following Dacie this series of cells are grouped under the heading of intermediate megaloblasts

Blood volume studies on the second patient were performed by the method of Gibson and Evelyn[7] as modified by Tysoe and Lowenstein[8]

Table I

BONE MARROW STUDIES
PERCENT OF TOTAL NUCLEATED RED CELLS

PATIENT NO 1

Day of Study (Fig 1)	Days After Start of		Megaloblasts %		Intermediate Megaloblasts %		Normoblasts %	
	ACTH	*B 12*	*Primitive*	*Mature*	*Primitive*	*Mature*	*Primitive*	*Mature*
3			50 0	31 5	5 5	6 5	1 0	5 5
8	3		21 6	34 2	13 5	16 0	7 1	7 0
13	8	0	11 0	30 0	5 0	19 0	6 0	29 0
15	10	2	4 5	23 0	2 5	30 0	9 0	32 0
20	15	7	0	8 0	3 0	5 0	7 0	77 0

Primitive—Pro and Basophilic Forms
Mature—Polychromatophilic and Orthochromatic Forms

The hematologic response is shown in Figure 1 There developed a gradual reticulocytosis which reached a peak of 14 6% on the ninth day of ACTH therapy This is equivalent to the expected rise[9,10] if the red cell count after the transfusion is taken as the initial level The slight increase of packed cell volume red cell count and hemoglobin may or may not be significant They could be attributed to shifts in plasma volume after ACTH

Because of the patient s poor condition 90 micrograms of Vita

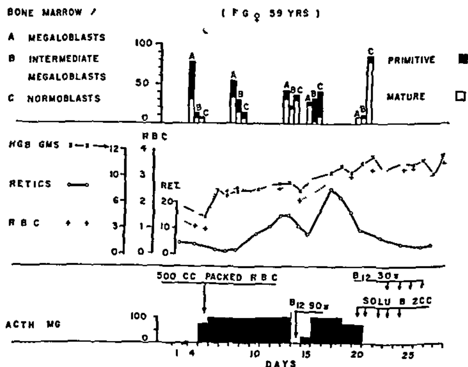


FIG 1

min B 12 concentrate were given intramuscularly 10 days after beginning ACTH therapy A prompt reticulocytosis developed with a peak of 25% sixty hours later ACTH was continued for 6 days after the administration of B 12 to a total of 1250 mgs in 15 days

ACTH produced the leukocytosis neutrophilia and eosinopenia characteristically found after the administration of this substance

The findings of five serial bone marrow aspirations are depicted in Figure 1 and Table I Primitive cells include the pro and basophilic forms and mature cells the polychromatophilic and orthochromatic forms *Before any therapy promegaloblasts and basophilic*

Table I

Patient No 1

Bone Marrow Studies
Percent of Total Nucleated Red Cells

Day of Study (Fig 1)	Days After Start of		Megaloblasts %		Intermediate Megaloblasts %		Normoblasts %	
	ACTH	*B 12*	*Primitive*	*Mature*	*Primitive*	*Mature*	*Primitive*	*Mature*
3			50 0	31 5	5 5	6 5	1 0	5 5
8	3		21 6	34 2	13 5	16 0	7 1	7 0
13	8	0	11 0	30 0	5 0	19 0	6 0	29 0
15	10	2	4 5	23 0	2 5	30 0	9 0	32 0
20	15	7	0	8 0	3 0	5 0	7 0	77 0

Primitive—Pro and Basophilic Forms
Mature—Polychromatophilic and Orthochromatic Forms

megaloblasts constituted over half of the total nucleated red cells and more mature megaloblasts another 30% Only 6 5% of the total nucleated red cells belonged to the normoblastic series The bulk of the remainder consisted of intermediate forms Many macrogranulocytes were also present

Under ACTH therapy the megaloblastic series showed partial maturation and there was a partial reversion toward normoblastic erythropoiesis This revision was never complete Although diminished in numbers many megaloblasts remained some of them promegaloblasts and basophilic megaloblasts A demonstrable increase in intermediate megaloblasts was observed consisting of both young and mature forms The numbers of normoblasts increased progressively On the fourth day of ACTH the normoblasts had increased from 6 5 to 14% and by the 11th day to 41%

After B 12 was administered a rapid change to normoblastic erythropoiesis occurred This was evident within 36 hours and complete on the seventh day

Case 2*

G T a 72 year old man was admitted to Queen Mary Veterans Hospital on January 6 1950 with the following complaints which had begun the previous summer Loss of appetite taste and 25 pounds weight right upper abdominal pain after meals fatigue and shortness of breath He had had no soreness of the tongue diarrhoea or neurological symptoms

He had been admitted to another hospital in 1941 for suprapubic prostatectomy for carcinoma of prostate on readmission in 1944 he was free of metastases In 1947 cataract extraction was performed at Queen Mary Veterans Hospital The family history was irrelevant

On physical examination there was pallor and faint icterus the tongue showed no atrophy the liver and spleen were not palpable There were no abnormal neurological signs There was no clinical or X ray evidence of metastases

The hematological values on February 13 were RBC 2 200 000 hemoglobin 9 0 gms hematocrit 31% MCV 141 cu m reticulocytes 3 0% WBC 5 700 platelets 132 000 The sternal marrow was megaloblastic

The general laboratory results follow Gastric analysis histamine fast achlorhydria Gastro intestinal radiography no lesion of the oesophagus stomach and duodenum Serum bilirubin (indirect) 2 0

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*Grateful acknowledgement is made to Dr L Guravich for his help in the clinical management to Miss R Numainville for her technical assistance and to Dr C Pick for the performance of blood volume in patient No 2

mgs % Serum protein 6 46 gms % albumin 3 07 globulin 3 39 Cephalin cholesterol flocculation negative Bromsulphalein test normal excretion Alkaline phosphatase 3 7 Shinowara Units acid phosphatase 0 1 Shinowara Units and glucose tolerance curve normal Total fecal fat normal with slight increase in combined fatty acids normal free fatty acids and slight decrease in neutral fat Chest X ray normal Electrocardiogram normal

During the first month of hospitalization the usual hospital diet was served to this patient this diet is relatively high in protein supplying approximately 90 gms of protein and 2 700 calories daily One month after admission and fourteen days before the first injection of ACTH he was placed on a daily intake of 2 700 calories containing 100 gms of protein The daily consumption of protein was measured and usually varied from 85 to 100 gms ACTH administration was begun on February 13 at the rate of 100 mgs per day for 3 days followed by 80 mgs for 2 days and finally 50 mgs for 3 days The total dosage was 600 mgs in 8 days The drug was given intramuscularly at 6 hour intervals

The patient s appetite had improved slightly during the control period and markedly during therapy The characteristic retention of fluids occurred with a 19 lb weight increase from February 10 to February 21 which was accompanied by swelling of the face edema of the legs basal rales in the right lung hepatomegaly splenomegaly and radiological enlargement of the heart shadow Consequently ACTH was discontinued on February 21 and with mercuhydrin a striking diuresis ensued

The hematological effects are shown in Figure 2 The reticulocytes reached a peak of 11 7% on February 22 9 days after starting treatment 16% being the anticipated peak [9]

During the following weeks the red cells hemoglobin and hematocrit gradually increased and reached a plateau early in March which was maintained until June There was the customary neutrophilic leukocytosis a brief lymphocytopenia and eosinopenia The prothrombin time and serum bilirubin were not affected by ACTH The total plasma proteins decreased from 7 17 gms % before treatment to 5 38 gms % during treatment and subsequently returned to the pre treatment range There was no significant alteration of the AG ratio The patient was in positive nitrogen balance before and after administration of ACTH and in negative balance during ACTH therapy

Gastric analysis was performed four times before once during and once after ACTH and on all occasions showed a histamine fast achlorhydria

By the beginning of June 4 months after ACTH the blood level

had clearly stabilized and while the remission was incomplete there was no definite indication of relapse Nor was there any evidence of gastrointestinal neurological or other clinical relapse Accordingly on June 9 he was given a single dose of B 12 120 micrograms by intramuscular injection A reticulocyte peak of 7 0% occurred 6 days later which exceeded the expected peak[9,10] *There was a further rise*

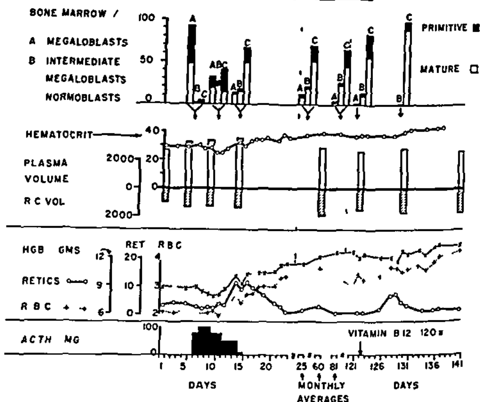


Fig 2

of the red cell count to 4 23 million the hematocrit to 44% and hemoglobin to 13 4 gms on June 28th

*During ACTH the total red cell volume increased from the pre*treatment average of 1185 ml to 1482 ml and subsequently rose to 1861 ml without further therapy (Figure 2) During this period the total hemoglobin *mass rose from 377 gms to 580 gms Comparable* values were obtained 7 and 18 days after the administration of B 12

The bone marrow (Figure 2 Table II) which was predominantly megaloblastic before therapy *showed a partial change towards nor*mal after ACTH i e there was a marked diminution in the number

Patient No 2

Table II

Bone Marrow Studies
Percent of Total Nucleated Red Cells

Day of Study (Fig 2)	Day After Start of		Megaloblasts %		Intermediate Megaloblasts %		Normoblasts %	
	ACTH	B 12	Primitive	Mature	Primitive	Mature	Primitive	Mature
(−35)			39 6	50 6	1 3	4 0	1 4	3 0
6			45 8	48 1	0	1 7	2 5	1 7
11	3		13 0	19 6	5 0	21 0	27 0	14 4
15	7		3 7	11 3	4 0	14 9	16 3	49 6
31	23		2 5	9 7	1 3	17 4	9 2	59 7
45	37		5 6	4 2	3 0	14 6	18 9	53 6
52	44		0 2	9 3	4 5	21 8	24 3	39 8
86	78		0 8	2 0	3 7	19 1	19 1	55 3
101	93		1 3	3 8	3 9	25 8	21 2	43 8
122	114		0	2 2	3 4	10 1	28 8	55 4
130	122	6	0	0	0 6	0	9 7	89 7

Primitive—Pro and Basophilic Forms
Mature—Polychromatophilic and Orthochromatic

of megaloblasts from 91% before therapy to 15% immediately after completion of ACTH therapy and to 5 1% three months later Intermediate megaloblasts increased from 1 7% before therapy to 18 9% immediately after ACTH and to 29 7% three months later Normoblasts increased from 4 2% before ACTH to 65 9% immediately after ACTH and were still 65% three months later Six days after the injection of B 12 megaloblasts had disappeared from the marrow and 99 4% of the nucleated red cells were normoblastic

GENERAL DISCUSSION

In two patients with pernicious anemia previously untreated short courses of ACTH induced a partial hematological remission Because these studies were part of a larger metabolic study in which the subjects received a high protein diet a control low protein diet was not provided for either patient Patient No 2 received a high protein diet for 6 weeks before the administration of ACTH the absence of hematological response during this period would indicate that this patient did not have nutritional macrocytic anemia Steatorrhea was excluded by the normal fat content of the stools and the normal oral glucose tolerance

That the hematological response to ACTH was incomplete is attested by the following the reticulocytosis was less than that expected after liver extract or Vitamin B 12 a second reticulocytosis occurred after B 12 which exceeded the expected response in the second patient there was a gradual and prolonged increase of erythrocyte count hemoglobin concentration red cell volume and total circulating hemoglobin after ACTH these values further increased after administration of Vitamin B 12

The changes in the bone marrow are of predominant interest After ACTH there was a partial reversion toward normal erythropoiesis This was manifest by a significant increase of normoblasts a marked decrease of megaloblasts and the presence of a considerable number of intermediate megaloblasts In patient No 2 after discontinuation of ACTH and until Vitamin B 12 was given the intermediate megaloblasts constituted 20–30% of all nucleated red cells Vitamin B 12 rapidly restored normal erythropoiesis

The cause of the partial hematological remission produced by ACTH remains speculative Spontaneous remission may be excluded ACTH is said to contain no Vitamin B 12 Can the same be said for animal protein factor ?

Recently evidence has accumulated to indicate that megaloblastic anemia may be produced by a quantitative but not necessarily an ab

solute deficiency of enzymatic factors involved in a chemical chain reaction which is necessary for the synthesis of nucleoprotein from amino nitrogen[11,12] That the deficiency need not be absolute would seem supported by the course of Addisonian Pernicious Anemia treated with Folic Acid [11,1,13,14,15,16,1] which produces hematological remission hypothetically by mass action effect until the tissues are completely depleted of Vitamin B 12 The failure of response of meg aloblastic anemia of pregnancy and the puerperium to refined liver extract and Vitamin B 12[1,19] [1] in some instances and the partial response to B 12 in other instances according to this hypothesis would be dependent upon the degree of deficiency of some substance required in the chain reaction other than Vitamin B 12 Similarly in our two cases of pernicious anemia ACTH may have mobilized small amounts of Vitamin B 12 theoretically still present in the tis sues and possibly also larger amounts of other factors which were not deficient and which by mass action may have enhanced the effect of the small amounts of available B 12

In view of the studies of Spiro et al [3] upon gastric pepsin and uri nary uropepsin it seems unlikely that administration of ACTH is followed by increased production of intrinsic factor in pernicious anemia although no direct tests were made upon the gastric juice of our two patients

SUMMARY

1250 mgms and 600 mgms of ACTH were given to two patients with pernicious anemia in relapse over a 13 and 8 day period respec tively Subsequently Vitamin B 12 was administered The hemato logical changes observed in the two patients were similar and showed the following (a) Reticulocytosis which was not optimal when com pared with the expected response after liver extract or B 12 therapy Subsequent treatment with Vitamin B 12 in both patients produced a second reticulocyte response which exceeded the anticipated maxi mum rise (b) An increase of the circulating red cell volume and total hemoglobin and an increase in the concentration of erythrocytes and hemoglobin in patient No 2 In patient No 1 the rise could not be fully observed because of the need for specific therapy However in the second patient there was a prolonged and gradual improvement which levelled short of the values later attained with B 12 In patient No 2 a macrocytosis persisted after ACTH but diminished after B 12 administration (c) A striking but not complete reversion of the meg aloblastic marrow towards normal after ACTH Completely normo blastic marrow appeared only after Vitamin B 12

CONCLUSION

Short term ACTH therapy produced a partial hematological remission of the blood and bone marrow of two patients with untreated pernicious anemia Subsequent treatment with B 12 resulted in complete remission

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DISCUSSION

DR JOHN W REBUCK What changes were observed in the pernicious anemia type of neutrophil?

DR LOUIS LOWENSTEIN The pernicious anemia type of neutrophil and the macrogranulocytes found in the bone marrow decreased in number but did not completely disappear after ACTH

CONCLUSION

Short term ACTH therapy produced a partial hematological re *mission of the blood and bone marrow of two patients with untreated* pernicious anemia Subsequent treatment with B 12 resulted in com plete remission

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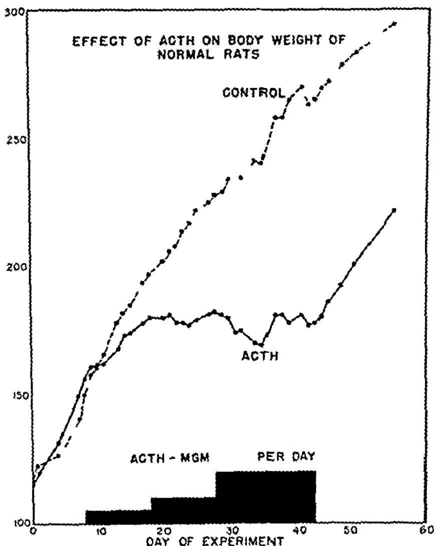


FIG 1 Rate of weight gain of rats treated with ACTH (Grams)

was a striking rise in neutrophils in every animal receiving ACTH as shown in Figure 4 This neutrophilia was transient disappearing rapidly when ACTH was discontinued and occasionally while the animals were still on treatment to levels equal to or lower than before ACTH was given

Two pigs received cortisone in doses of 100 mg per day No significant change was noted In one animal an infection was produced by injecting a culture of virulent staphylococcus Another was treated with folic acid In both instances leukocytosis and neutrophilia oc

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The Effects of ACTH and Cortisone on the Blood of Experimental Animals*

J G Palmer,† G E Cartwright and M M Wintrobe

COLLEGE OF MEDICINE UNIVERSITY OF UTAH SALT LAKE CITY

This report describes the results of a study of the effects of ACTH and cortisone on the blood of normal animals and of animals with various induced hematologic disorders

Male albino rats (Sprague Dawley) weighing approximately 100 grams were used A few observations were made on swine All hematologic studies were made on freely flowing tail vein blood ACTH was administered subcutaneously every six hours but all doses are recorded as the total daily dose expressed in terms of Armour standard I a 1 A The total daily dose of cortisone was administered intramuscularly in two divided doses every 12 hours

RESULTS

The effects of ACTH administration to normal rats are shown in Figures 1 2 and 3 each curve representing the mean of a group of 5 rats On a dose of 2 mg per rat slight impairment of growth and transient eosinopenia occurred (Figure 2) These changes became more pronounced as the dose was increased but definite neutrophilia and lymphocytopenia were not seen until a dose of 8 mg was reached When the drug was discontinued there was rapid weight gain and a rise in eosinophils and lymphocytes above control levels while the neutrophils decreased to control values At no time was any significant change in hemoglobin or reticulocytes noted (Figure 3)

ACTH was administered in amounts of 50–100 mg per day to several Chester White swine which were anemic and leukopenic as a result of a deficiency in pteroylglutamic acid the deficiency having been produced by feeding a purified diet containing succinylsulfathiazole and a folic acid antagonist as described elsewhere [1] There

* This study was supported by a grant from the Damon Runyon Memorial Fund

† Fellow American Cancer Society

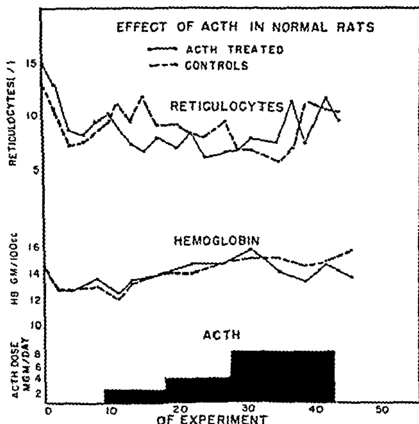


Fig 3 The effect of ACTH on hemoglobin and reticulocytes in rats

in the treated animals than in those receiving aminopterin alone No significant difference in the degree of leukopenia was noted although the ACTH treated group tended to have lower leukocyte counts than the others These results are shown in Table I

The course of the leukocyte counts of the rats made chronically

Table I

Effect of ACTH and Cortisone on Leukemia Due to Aminopterin

	WBC × 1000/c mm	
	Initial	*Final*
Aminopterin	21 4	6 8
Aminopterin & ACTH (8 mg/day)	23 2	2 1
Aminopterin & Cortisone (5 mg/day)	20 8	8 3

curred but the degree of change was not as great as that seen after ACTH administration No change in the red cells was noted other than slight *reticulocytosis in the ACTH treated animals All of the* ACTH treated animals appeared more ill afterwards than before ACTH was given

In order to study further the effect of ACTH and cortisone in de

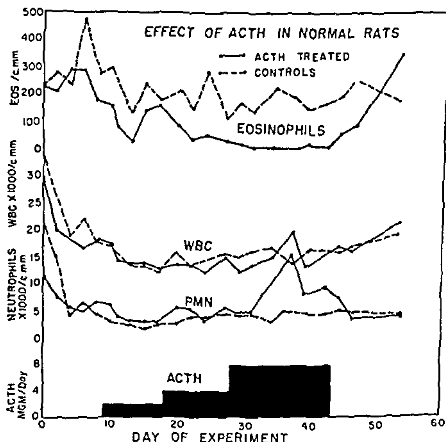


Fig 2 The effect of ACTH on leukocytes in rats

ficient animals acute and chronic PGA deficiencies were produced in rats the acute deficiency by giving aminopterin and the chronic de ficiency by dietary methods similar to those used in the swine

In studying the influence of an acute deficiency of folic acid the administration of ACTH or cortisone was begun 24 hours prior to the giving of aminopterin and the latter was continued for 3 to 5 days Neither ACTH nor cortisone prevented the diarrhea weight loss or leukopenia which develop when folic acid deficiency is pro duced actually symptoms of toxicity appeared 12 to 24 hours sooner

of deficiency progressed much more rapidly and all of the animals died within two days

The remainder of the animals were observed until the 60th day at which time the deficiency had become well established They were then divided into four groups of ten animals each One group was treated with ACTH 8 mg day a rapid fall in the number of leuko cytes occurred with progression of the signs of deficiency One group was treated by removing the succinylsulfathiazole and pteroylglutamic

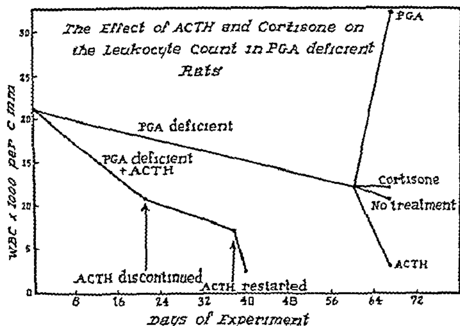


Fig 5 The effect of ACTH cortisone and folic acid on leukocytes in folic acid deficient rats

acid (PGA) antagonist from the diet and adding PGA 7 5 mg/kilo gram of diet rapid recovery occurred with the leukocyte count rap idly rising to very high levels One group treated with cortisone 5 mg/day showed no change as did the group receiving no treat ment

Slight anemia developed in these animals by the 60th day with hemoglobins ranging from 10 5–12 5 grams/100 ml the normal range in this laboratory being 14 5 to 16 5 grams/100 ml No change was noted in the seven days during which they were treated

The effects of ACTH and cortisone in experimentally produced hemolytic anemia are shown in Figure 6 An anti rat red cell rabbit

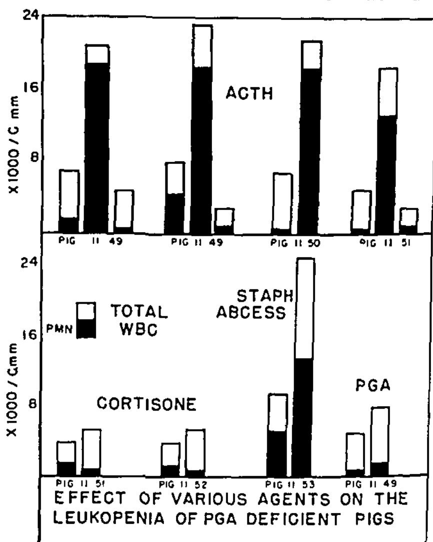


FIG 4 The effect of cortisone infection folic acid and ACTH on leukocytes in folic acid deficient swine

ill is presented diagrammatically in Figure 5 Fifty rats were placed on a purified diet containing succinylsulfathiazole and a PGA antagonist (crude methyl folic acid) One group of ten rats was treated with ACTH 8 mg/day from the time the diet was started and it may be seen that leukopenia developed more rapidly in these animals than in the untreated group they also lost weight and appeared ill sooner ACTH was discontinued after 21 days and although no improvement was noted progression of the deficiency was less rapid When ACTH was restarted on the 38th day leukopenia and signs

appeared to prevent the development of anemia The difference illustrated in the figure was found to be statistically significant The most pronounced effect however was on the leukocytes In the case of the animals given the larger dose of anti serum the ACTH treated animals developed less leukocytosis than the control animals With the smaller dose since no anemia developed in the treated animals leukocytosis would not necessarily have been expected It may be seen that none occurred except on one day in the ACTH treated group

The number of nucleated red cells and reticulocytes in the blood was slightly higher in the ACTH treated group receiving the larger dose of anti serum than in the controls but the difference was not significant

In Table II are shown the means of the leukocyte counts in rats

Table II

EFFECT OF ACTH AND CORTISONE ON LEUKOCYTOSIS ASSOCIATED WITH INFLAMMATION DUE TO TURPENTINE

	No of Rats	*WBC × 1000/c mm*
Turpentine	12	36 8 ± 1 2*
Turpentine & ACTH	10	20 4 ± 0 8*
Turpentine & Cortisone	8	16 4 ± 0 6*

* Mean ± Standard Error of Mean

which were given repeated injections of turpentine with the object of producing a severe inflammatory reaction It was found that animals receiving ACTH and cortisone did not develop the degree of leukocytosis seen in those receiving turpentine alone No anemia developed in any of the animals

Because these observations suggested that under a variety of experimental conditions the principal effects of ACTH and cortisone on the blood of rats and swine were on the leukocytes and because it seemed as if the effects of ACTH and cortisone might not be identical additional experiments were carried out

ACTH and cortisone were administered to intact and to adrenalectomized rats three to five days postoperatively The results are illustrated in Figure 7

Sixty intact rats were used ACTH was given in a single injection of 5 mg to 35 rats cortisone in a single injection of 5 mg to 25 rats Leukocyte counts were made six hours after the injection In the figure the means are shown It will be seen that the administration of ACTH resulted in an increase in the total leukocyte count due to an increase in neutrophils whereas cortisone had the opposite effect

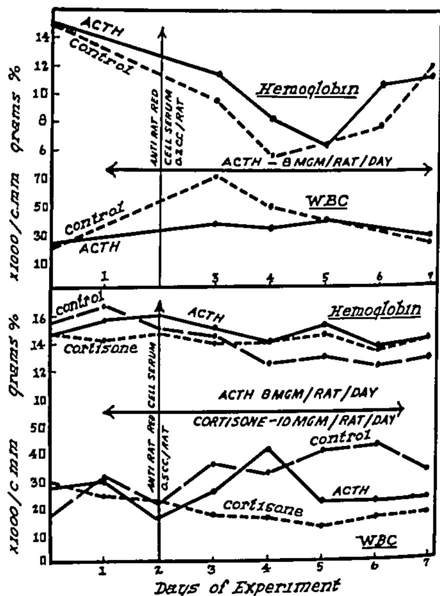


Fig 6 The effect of ACTH and cortisone on hemolytic anemia in rats due to anti rat red cell rabbit serum

serum was prepared by repeatedly injecting washed rat cells into rabbits The serum was removed and injected into rats intraperitoneally The regular injection of ACTH 8 mg/day or of cortisone 10 mg/day was begun prior to the administration of the anti serum

It may be seen from Figure 6 that when a relatively large quantity of anti serum was given ACTH did not prevent the development of the anemia With a smaller dose however both ACTH and cortisone

bility exists that adrenal rests might be present on which ACTH may be acting Such rests have been found around the testicles therefore combined adrenalectomy and orchidectomy were performed in a group of ten rats and they were placed on cortisone 5 mg/day ACTH administered to these animals produced effects identical with the animals which were simply adrenalectomized

Since ACTH is a protein it was also decided to see if neutrophilia in adrenalectomized cortisone maintained animals would be produced by other proteins Consequently several bovine plasma fractions and a suspension of purified casein were injected into such animals No significant change in the leukocytes was noted

GENERAL DISCUSSION

Considerable work has been done by others on the effect of ACTH and cortisone on the blood cells but principal attention has been focused on the lymphocytes [6,7] and eosinophils[3,5,10] Neutrophilia has been known to occur and has been reported to be present in adrenalectomized mice given ACTH and in patients with Addison s disease[5] It has been reported that ACTH produces polycythemia with extramedullary blood formation in mice[6] No effects on platelets have been noted[8] In this study lymphocytopenia eosinopenia and neutrophilia were observed in normal animals following the administration of ACTH but no changes in red cells were noted Platelets were not studied

In PGA deficiency Dougherty and Dougherty[9] found that the effects of aminopterin on lymphatic tissues and lymphocytes in mice were decreased by adrenalectomy They concluded that the effects of aminopterin on lymphatic tissues are mediated in part through the adrenals Our own observations are of interest in that adrenal cortical stimulation apparently increases the toxicity of PGA deficiency suggesting thereby that the activity of PGA antagonists is modified in the tissues by cortical hormone These results are not in disagreement with those of Dougherty and Dougherty since they found that the effect of aminopterin on myelopoiesis took place in adrenalectomized animals and since no detailed observations were made on lymphocytes in our study

It has been reported[11] that improvement may take place following administration of ACTH in patients with acquired hemolytic anemia presumably due to anti red cell antibodies The hemolytic anemia produced in this study which may be comparable to the human disorder was also favorably influenced when the process was mild The most striking effect however both in the instances of hemolytic anemia and in the experimentally produced sterile inflam

The adrenalectomized group consisted of 14 rats These were maintained on 1 per cent NaCl given in the place of drinking water from the time of operation Twenty seven animals received ACTH 17 cortisone Doses and time intervals were the same as in the intact group Adrenalectomy was associated with an increase in the number of both neutrophils and lymphocytes It will be seen that the administration of cortisone reversed these changes ACTH on the other hand produced a further rise in neutrophils

These results suggested that with respect to the neutrophils

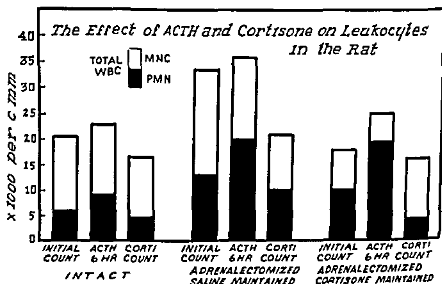


FIG 7 The effect of ACTH and cortisone on leukocytes in intact adrenalectomized saline maintained and adrenalectomized cortisone maintained rats

ACTH and cortisone act differently and that this effect of ACTH is not mediated by the adrenal gland To elucidate this further ACTH was administered to adrenalectomized animals while they were receiving cortisone Seventy rats were adrenalectomized and were given cortisone 5 mg/day Three to five days after operation ACTH was given and a striking rise in neutrophils occurred Cortisone had the opposite effect as it did in the adrenalectomized saline maintained animals On prolonged administration it was found that the marked neutrophilia which follows ACTH was not maintained under any of these conditions It could however be produced twice in the same animals by repeating single injections at 5 day intervals

Although these results suggest that the neutrophilia producing effect of ACTH was not mediated through the adrenals the possi

bility exists that adrenal rests might be present on which ACTH may be acting Such rests have been found around the testicles therefore combined adrenalectomy and orchidectomy were performed in a group of ten rats and they were placed on cortisone 5 mg/day ACTH administered to these animals produced effects identical with the animals which were simply adrenalectomized

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mation due to turpentine injection was suppression of leukocytosis The mechanism of this action has not been clarified by our studies

Neutrophilia following the administration of ACTH occurred in intact animals in pteroylglutamic acid deficient swine and in adrenalectomized animals This was true in the adrenalectomized animals even though they were receiving cortisone in greater than maintenance doses Several possible explanations may be offered for this

1 ACTH stimulates adrenal rests which have not been completely removed If small remnants of adrenal tissue were still present in these animals it would seem likely that they were stimulated maximally If they were not the adrenal hormone secreted would have had to have an effect not shared by cortisone since under all the conditions it was used in our studies cortisone did not produce a significant neutrophilia Neutrophilia has also been produced by ACTH in animals maintained on cortisone and adrenalectomized and orchidectomized in order to eliminate any rests which may be present around the testicles Neutrophilia did not result from the administration of cortical extract to adrenalectomized cortisone maintained animals

2 The neutrophilia may be a non specific effect produced by foreign proteins in the preparation The other proteins used had no influence on the leukocytes This of course does not rule out the possibility that the neutrophilia is the result of the action of a foreign protein It would appear however that the action must be a specific one produced by certain substances and not by others

3 The reaction may be an extra adrenal effect of either ACTH or some other substance present in the preparations Neutrophilia in adrenalectomized cortisone maintained animals has been produced by five different lots of ACTH (all that have been tried) ranging in potency from 25 per cent of standard to 22 times standard Undoubtedly in all of these preparations most of the material was inert as far as adrenocorticotrophic activity is concerned It is of interest that the injection of a preparation of melanophore hormone was followed by a definite *neutrophilia under these conditions It would seem then* that the most likely explanation for the neutrophilia would be that it is produced by some substance not necessarily having ACTH activity found in preparations from the pituitary gland Admittedly however this hypothesis is not proven by these studies

SUMMARY

1 In intact rats the administration of ACTH resulted in impaired growth transient neutrophilia lymphocytopenia and eosinopenia The administration of cortisone was followed by lymphocytopenia and eosinopenia but *not by neutrophilia*

2 The administration of ACTH to PGA deficient swine resulted in marked transient neutrophilic leukocytosis whereas cortisone did not have this effect In PGA deficient rats ACTH apparently accelerated the production of the deficiency

3 In rats ACTH and cortisone prevented the development of a mild hemolytic anemia produced by the injection of small doses of an anti rat red cell rabbit serum but did not prevent anemia when the dose was larger Both hormones suppressed the leukocytosis accompanying the hemolytic reaction as well as the leukocytosis of inflammation

4 Neutrophilia produced by ACTH occurred in the absence of the adrenals even though the animals were maintained on adequate doses of cortisone It is suggested that the neutrophilia may have been produced by a substance or substances in the pituitary preparations which does not act through the adrenals

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DISCUSSION

DR ROBERT S SPEIRS I was very interested in Dr Palmer s comments on the comparison of tail and heart blood samples and I would like to put in a word for the use of tail blood In our laboratories we have used this method of taking blood for three years and find it to be a repeatable and accurate procedure However a number of very important precautions should be taken If the animals are not adrenalectomized some strains of mice will following a very slight disturbance show tremendous changes in the number of blood cells In the C_{57} Brown mice for example the movements of an animal caretaker near the cage is sufficient to produce an eosinopenia In order to standardize their environment we designed a closed cabinet in which the cages are placed This cabinet enables us to keep the mice in the dark at a constant temperature of 80° F right in the laboratory where we perform the blood counts (See Fig 8)

When the equipment and solutions are ready the mice are taken out of the cabinet and placed in a jar under a 100 watt lamp Within a few minutes they become active and a blood count is taken immediately Less than 15 minutes is involved from the time the mice are first disturbed until a sample of blood is taken Only free flowing blood is used as milking of the tail can lead to very inaccurate counts The standard error of the procedure is determined by taking 4 pipettes of blood from the same mouse within a period of two minutes and calculating the number of cells A difference in the count of cells from each pipette will include not only the mechanical error and error of counting but also the error due to variations in the number of cells in different drops of blood In our laboratory the standard error was found to be 1 2 times the square root of the number of cells counted

It should be strongly emphasized that there are tremendous differences between strains of mice In our report yesterday we indicated



Fig 8

that in the special hybrid mice we were using as little as 6 micrograms of cortisone would produce an 80 percent decrease in the number of circulating eosinophils of adrenalectomized mice In another strain the Jax 129 mice as much as 96 micrograms of cortisone were necessary to produce the same results This difference in sensitivity undoubtedly applies to ACTH and other hormones also I think that it is important in experiments of this nature to use inbred strains of mice or their hybrids Coming from a genetics laboratory you would expect me to say that

As far as sex differences are concerned most of our work has been performed on males The females will respond but for some reason there seems to be a great deal more variation We are undertaking a study at the present time to see whether it can be alleviated by testosterone

DR EDWARD H KASS We have had similar experiences to those of Dr Palmer and his associates in our studies of the effect of ACTH on the leukocytes of white mice Several difficulties arose during our attempts to determine an adequate dose of ACTH to use in experiments with mice The first is that repeated bleeding of the same mouse is a form of severe stress as has been shown by Speirs and Meyer Inasmuch as the mouse has a total blood volume of 1 5–2 0 ml it is understandable that loss of even a few drops of blood induces severe anemia It is therefore essential that each animal be bled but once and that large numbers of mice be treated under standardized conditions to obtain useful data

Secondly as Speirs and Meyer have shown handling mice is sufficient stimulus to induce eosinopenia for several hours We have been able to show that the injection of saline gelatin or gamma globulin intraperitoneally into mice may lead to eosinopenia which may last for as long as six hours Thirdly if venous blood from the end of the tail is used the leukocyte and eosinophile counts may be as much as three times as great as in arterial blood This difficulty may be avoided either by heating the entire animal or its tail (and we have felt this procedure constituted a stress of considerable degree) or by doing ventricular punctures or by lightly anesthetizing the mice and cutting off the tail very close to the body so that arterial and free flowing venous blood are collected If the latter procedure is done rapidly no effect on eosinophiles or leukocytes is observable

Using these precautions we found that 1 25–2 5 mg of ACTH were required to maintain eosinopenia for a period longer than the 6–8 hours of eosinopenia which may be induced by nonspecific proteins When a dose of 2 5 mg of ACTH in a volume of 0 5 ml was given intraperitoneally every 12 hours eosinopenia and leukopenia were maintained for about 6 days after which the values returned to normal levels despite the repeated administration of this very large dose of hormone The latter observation indicates that the dose used although equivalent to about 8 grams per day on a weight basis in the human adult is not toxic to mice The Tumblebrook Farm strain has been used in all of these studies

Eosinopenia in the mouse is frequently accompanied by leukopenia and the latter in turn is due in large part to the lymphopenia which is induced by ACTH The mouse normally has 60–

80% lymphocytes so that the transient polymorphonuclear leuko cytosis which is induced by ACTH is overcome by the more marked lymphopenia which occurs after injection of the hormone

We feel that these observations are of interest because if one ex amines the literature in which mice have been used in experiments dealing with ACTH there are very few instances in which doses of the hormone have been used which exert effects greater than those induced by nonspecific stimuli

DR EMANUEL B SCHOENBACH I was quite interested in this discussion and I would like to speak about Dr Kass remarks We do hundreds of white blood cell counts on mice from the tail vein and get quite reproducible results It is part of the study we have been conduct ing with various drugs in neoplasia We use the tail veins and al though the counts may be higher than those from heart blood we find that the latter procedure also introduces variables when one anesthetizes the mouse to get the heart blood etc

I agree with Dr Kass that the amount of ACTH one may give a mouse without any observable changes is really tremendous We have employed a dosage of 80 milligram equivalents daily in mice and see very little change in the thymus the nodes or the spleen This is in contrast to our experience with cortisone with which we ob serve diminution of the size of the spleen and the thymus with reg ularity

Now the relationship of aminopterin to cortical hormones or hormones that stimulate the adrenal cortex is really quite interest ing We found several years ago that there was a difference in toler ance between female and male mice to aminopterin Females tolerate approximately twice as much as males We have conducted experi ments with various hormones orchiectomy and oophorectomy but could not alter the sex difference until we performed a bilateral adrenalectomy

The effect of ACTH and aminopterin was investigated in mice and as has been reported at this Conference we could find no bene ficial effect from the administration of ACTH or cortisone together with the aminopterin as measured by reduction in toxicity or pro longation of life in mice bearing a transplantable leukemia

DR J G PALMER I would like to say in all our studies we used tail vein blood We have not been able to find any significant difference between tail vein blood and heart blood in animals which have been anesthetized with nembutal or with ether

PART VII

FUNDAMENTAL ACUTE INFLAMMATORY REACTION INFECTION, IMMUNOLOGY AND HYPERSENSITIVITY

43

The Action of ACTH and Cortisone on Experimental Ocular Inflammation*

Alan C Woods and Ronald M Wood

WILMER OPHTHALMOLOGICAL INSTITUTE OF THE JOHNS HOPKINS HOSPITAL AND JOHNS HOPKINS UNIVERSITY MEDICAL SCHOOL BALTIMORE

In other communications experiences with ACTH and cortisone in the treatment of various eye diseases were outlined It was found that when these agents were injected parenterally they had a profound effect in controlling the inflammatory and exudative phases of such eye diseases as non granulomatous iritis sympathetic ophthalmia and tuberculous uveitis It was also reported that the local or topical use of cortisone in the eye produced a therapeutic effect quite comparable to that noted after the systemic injection of ACTH or cortisone

At the time the clinical investigation was begun various experiments were performed in rabbits These experiments were designed to determine the action of these agents on various forms of ocular inflammation The eye has certain distinct advantages as an organ in which to study the course and progress of inflammation permitting an exact evaluation of the degree of ciliary congestion edema and inflammation of the iris exudation in the anterior chamber and the secondary changes of edema infiltration and vascularization of the cornea

* The ACTH used in these experiments was supplied by The Armour Laboratories The cortisone used was purchased by a grant from the Public Health Service National Institutes of Health

Two untreated rabbits sensitized to horse serum showed marked ophthalmic and cutaneous reactions to the anterior chamber and intracutaneous injection of horse serum Two similar rabbits were treated with ACTH 48 hours before and 48 hours after inoculation One of these rabbits showed complete blocking of both the ophthalmic and the cutaneous reactions The second an extremely sensitive rabbit with a titer of 1 12 800 showed a partial blocking of the ophthalmic reaction but no blocking of the cutaneous reaction Two other hypersensitive rabbits were treated with cortisone acetate for 96 hours prior to inoculation and 48 hours after inoculation Both of these rabbits showed complete blocking of the ophthalmic reaction but only partial blocking of the cutaneous reaction

In a quantitative evaluation of cortisone it was observed that all eyes of four untreated sensitized rabbits reacted with a violent iritis of eight days duration to the anterior chamber injection of 0 1 cc undiluted horse serum In four rabbits receiving 10 mgms cortisone for four days prior to anterior chamber injection and seven days after such injection this inflammatory reaction was completely blocked in three animals and almost completely blocked in a fourth In similar animals receiving 5 mgms of cortisone the reaction was partially blocked and of short duration In animals receiving 2 5 mgms cortisone there was decidedly less blocking reaction the majority of the eyes showing moderate reactions but of comparatively short duration

It is quite evident a daily dose of 10 mgs has an almost complete blocking effect on the inflammatory phase of the ophthalmic protein reaction and that smaller amounts of the hormone have a comparatively lesser action

The cutaneous reaction in rabbits sensitized to Alpha streptococci was delayed by ACTH administered 48 hours both before and after inoculation but was not blocked Cortisone acetate administered 96 hours prior to inoculation and 48 hours after inoculation almost completely blocked the cutaneous reaction in two rabbits so treated

Control untreated rabbits sensitized to killed beta streptococci showed well marked ophthalmic and cutaneous reactions to the anterior chamber and intra cutaneous injection of the specific streptococcus antigen In similar sensitized and injected rabbits treated with cortisone prior and subsequent to the test injections the ophthalmic and cutaneous reactions were completely or partially blocked The degree of blocking appeared somewhat proportionate to the degree of pretreatment sensitivity

Treatment with ACTH and cortisone in immune allergic rabbits prior to a shocking dose of tuberculin almost completely blocked the inflammatory focal reaction in eyes with a secondary tuberculosis

In both ACTH and cortisone treated animals this blocking effect completely disappeared about two weeks after cessation of treatment In rabbits in whom cortisone was continued for three weeks the treatment appeared to block the inflammatory phase of the tuber culous process completely but had no effect on the actual progress of the tuberculous lesions

These four experiments clearly indicate that in the experimental rabbit both ACTH and cortisone have the ability to block the in flammatory phase of the allergic reaction The question immediately arises whether this ability to block ocular inflammation is confined to inflammation secondary to the hypersensitive reaction or whether it is similarly manifested in inflammation not related to allergy This point was investigated by determining the ability of cortisone to block inflammation secondary to irritants

The injection of 10 mgms cortisone a day four days prior to anterior chamber injection and five days after such injection com pletely blocked the inflammatory reaction due to the introduction of glycerin in the anterior chamber Likewise the systemic injection of 10 mgms of cortisone daily for four days prior to the anterior chamber injection of jequirity infusions completely blocked the in flammatory reaction shown by the controls When cortisone was dis continued the previously treated rabbits showed a mild inflamma tory reaction of several days duration

Since both ACTH which acts through the stimulation of the adrenal cortex to produce endogenous adrenal cortex hormones and exogenous cortisone administered parenterally have a similar effect in blocking the inflammatory phase of the hypersensitive reaction it seems a logical conclusion that cortisone *per se* is at least one if not the most important of the adrenal cortex hormones responsible for this effect The question therefore immediately arises as to whether this action of cortisone is a direct one on the tissue cells or is an in direct one through some intermediary agent

In experiments to determine whether cortisone topically applied had any effect on control of the inflammatory reaction it was ob served that the anterior chamber injection of 0 25 mgms of cortisone synchronously with an intoxicating injection of 0 1 c c horse serum resulted in a partial blocking of the inflammatory phase of the ana phylactic protein reaction in sensitized rabbits The injection of 1 25 mgms cortisone synchronously with the horse serum resulted in a slightly greater blocking effect in similarly sensitized rabbits

The anterior chamber injection of 1 25 mgms of cortisone com pletely blocked for two days the inflammation which normally fol lows the injection of 0 1 c c of a 1 4000 dilution of jequirity infusion It almost completely blocked for a period of twenty four hours the

reaction which follows the injection of a similar amount of a 1 2000 dilution Thereafter the blocking effect of the anterior chamber injection of cortisone disappeared and ten of the twelve eyes developed a delayed inflammatory reaction Cortisone injected in the anterior chamber three days before the injection of jequirity infusions had no effect whatsoever on blocking the inflammatory reaction

COMMENT

The experiments here reported confirm the clinical observations reported elsewhere—that ACTH and cortisone have a profound effect on certain ocular inflammatory and exudative reactions It seems quite clear that both ACTH and cortisone administered parenterally can block the inflammatory and exudative phase of the anaphylactic protein reaction in the eye as well as that of the allergic reaction due to bacterial hypersensitivity and of the focal reaction produced in tuberculous eyes by the systemic injection of tuberculin this latter being another example of bacterial allergy The first and most natural assumption is that in some way these agents inhibit or influence the antigen antibody reaction The experiment here reported showing that ACTH and cortisone block the inflammatory reaction due to irritants equally as well as they block the inflammation secondary to the hypersensitive reaction is evidence that their action in blocking inflammation is independent of any effect on the antigen antibody reaction

SUMMARY

1 Cortisone and ACTH injected parenterally have a profound effect on the inflammatory phase of the hypersensitive reaction blocking the inflammation and exudation which occurs in the ocular protein anaphylactic reaction the ophthalmic reaction secondary to bacterial allergy and the focal reaction in tuberculous eyes

2 Cortisone and ACTH block effectively the inflammatory reaction in the eye produced by irritants such as glycerin or jequirity infusion

3 Cortisone injected locally in the anterior chamber has an immediate but temporary effect in blocking the inflammatory phase of the protein anaphylactic reaction in the eye and the inflammatory reaction secondary to the anterior chamber injection of glycerin and jequirity

4 The accumulated clinical and experimental evidence of the effect of cortisone on ocular inflammation suggests that its effect may be due to a direct action on the local mesenchymal tissue

DISCUSSION

DR HANS SELYE In confirmation of what Dr Harvey has said about the anti inflammatory action of these compounds I would like to add that we have injected a dilute solution of formaldehyde into the joint regions of rats and have found that this causes a very marked fibroblastic reaction

I think many of those who commented on this particular reaction had a feeling that it is an abscess formation We studied a number of irritants before we arrived at formaldehyde because it does not tend to cause real abscess formation It produces a very cellular fibroblastic granuloma which seems to be closer to the rheumatoid type of reaction

For those interested in experiments along these lines I may add as a technical point that our co worker Dr Coutu by coincidence has found that if you give U S mustard powder it is even more effective in producing the desired reaction—that is to say—one which is not associated with much suppuration Perhaps the granular nature of the irritating material in mustard powder (which can be given as a 10% suspension) is responsible for this particular type of cellular response

All the gluco corticoids which we have so far assayed including cortisone dehydrocorticosterone and corticosterone have an inhibitory reaction upon this reaction to the local irritation of the joint The inhibition can be so pronounced that you see such an irritating substance as mustard powder under the skin where you inject it with no edema surrounding it while of course unprotected animals develop a marked reaction

The counterpart to this is obtained with desoxycorticosterone which greatly augments the inflammatory response to the same type of irritant Here again of course there is no immunologic or allergic reaction at stake

ACTH and Leukocytic Performance in Windows in Man*

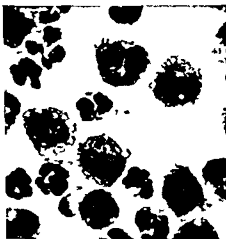
John W Rebuck, Richmond W Smith, Jr and R R Margulis
THE HENRY FORD HOSPITAL DETROIT

Exudative cells in acute inflammation in man were studied in serial preparations obtained in chronological sequence from individual lesions in the manner of our previously described[1,2,3] human windows The preparations were stained like blood smears Antigens both non pyogenic and non reproducing (diphtheria toxoid egg white and old tuberculin) were utilized In healthy volunteers control lesions were characterized by an early mild histiocytic and pronounced neutrophilic response for 8 hours which gave way in the next 4 hours to a peak lymphocytic response (Fig 1A) This lymphocytic response was followed by lymphocytic hypertrophy at 12 to 14 hours and culminated in an almost purely histiocytic picture at 16 to 24 hours (Fig 1B) This cytologic picture of the inflammatory cycle is similar to that originally described in experimental animals by Metschnikoff[4] and later fully confirmed by Maximow Downey Kolouch and Dougherty

This succession of (a) local histiocytic response possibly reinforced by monocytes followed in turn by (b) neutrophils (c) lymphocytes (d) hypertrophying lymphocytes and the subsequent pronounced (e) histiocytic picture we have termed a complete or major cellular response In a study of the response to the trauma of the technic alone the cellular exudate merely consisted of the first two cell types local histiocytes and neutrophils following which the lesion healed This type of response we have designed as incomplete or minor as to exudative cell type It should be remarked however that at times this type of cellular defense terminates in an eminently successful outcome for the patient healing The danger of interpretation lies in the fact that this is also the type of cellular exudate that can be seen in response to a virulent agent capable of overcoming local defenses and leading to septicemia

* The pituitary adrenocorticotrophic hormone employed in these studies was made available through the cooperation of The Armour Laboratories
We are indebted to Dr E L Whitney and his associates in the Division of Ophthalmology f erous help proffered in this study

Forty two individual lesions were so studied in twelve patients both prior and subsequent to the administration of ACTH and in controls A variety of clinical syndromes was present in these patients and included acute and chronic ocular inflammatory disease malig nant ocular disease degenerative ocular disease Boeck s sarcoid rheumatoid arthritis periarteritis nodosa congenital syphilis and aplastic anemia The ophthalmological studies and general clinical aspects of this group of patients have been reported in detail else where by Olson and his associates [5,6]



A

Fig 1A Normal control 12 hrs inflammation in man after diphthe ria toxoid Lymphocytes and de generating neutrophils x 1800



B

Fig 1B Normal control 20 hrs inflammation in same lesion as 1A Note predominance of histiocytes x 1800

The inflammatory response to the experimental lesions in pa tients studied prior to ACTH it must be emphasized did not often correspond to the inflammatory response found in healthy volunteer control (Figs 1A B) Thus in affected patients a stimulus normally non pyogenic elicited a pyogenic response as depicted in Fig 2 or conversely a non pyogenic stimulus could elicit an abnormally pre dominant histiocytic response prior to administration of the hormone which in some instances could be correlated with the clinical disease already present This deviation from the normal of the pre treatment response in general determined the nature of the cellular reaction which occurred during the period of hormonal administration For example Fig 3 illustrates the histiocytic conversion of the inflam matory exudate at 12 hours of inflammation after therapy in the

same patient who originally responded with the pyogenic picture presented in Fig 2

In patients with an essentially normal mesenchyme there was *often no apparent change in the qualitative cellular reaction during* the period of treatment Occasionally (one window) however there was a depression of lymphocytic participation at the customary 8 to 12 hour period As will be discussed below this latter finding is of the greatest significance and is illustrated in Fig 1 which should be *compared with Figs 1 A and 7 Occasionally (two windows) there was*



Fig 2 Case of chronic uveitis with bullous keratitis Pretreatment 12 hrs of inflammation Pyogenic response to a non pyogenic antigen Note lymphocytic hiatus only a single lymphocyte present in this representative field x 2000



Fig 3 Same case as Fig 2 after treatment 12 hrs of inflammation Conversion of response to a largely histiocytic content Two neutrophils are near center of field x 2000

an inhibition of neutrophilic phagocytosis of India ink (Fig 5) in contrast to pre treatment controls In addition there was much less over all phagocytosis in the local area of inflammation because of the marked diminution in the lymphocytes hypertrophying lymphocytes and their resultant histiocytes all of which were cell types with marked phagocytic ability in the pretreatment controls

The most frequent response with therapy of the group with chronic diffuse inflammatory lesions with granulomatous lesions and with diffuse hypersensitivity disease was characterized by a dropping out of the lymphocytes at the 8 to 12 hour period (Fig 4) and consequently of the hypertrophying lymphocytes as well at the 12

to 14 hour period The most frequent response with the group then was one which we have designated above as n or minor As a result of this near lymphocytic hiatus was dominated by neutrophils and histiocytes the latter diminished numbers The important exception was the Boeck s sarcoid (Fig 6) which consisted almost exclusivel of histiocytes at an unprecedented 5¾ hour stage

Four windows applied to a patient with degenerati



Fig 4 Case of retinitis pigmentosa Typical lymphocytic hiatus after ACTH Lymphocytes and hypertrophying lymphocytes have dropped out leaving scattered neutrophils and histiocytes to form the bulk of the cellular content at 12 hrs inflammation Compare with Fig 1A and Fig 7 x 2000

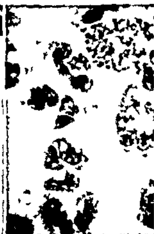


Fig 5 Case of sq carcinoma of limbus inflammation Sparse phagocytosis of India ACTH See text for c lymphocytic hiatus he cytic lymphocyte is al x 2000

bilateral chorioretinitis lacking clinical response in th ACTH revealed far reaching alterations in verdoperoxid nisms Previously we have described[7] in the normal th ment of verdoperoxidase in migrated neutrophils in the fi inflammation followed by the assumption of peroxida cytoplasm by the lymphocytes and hypertrophying lym well as the histiocytes A partial explanation for this find phocytes may be found in their phagocytosis of shed bits philic cytoplasm In the pretreatment windows the r lymphocytes and hypertrophying lymphocytes (Fig 7)

abundant verdoperoxidase In the two windows studied subsequent to therapy in the patient under discussion all the manifestations of verdoperoxidase were significantly inhibited (Fig 8)

Not only was verdoperoxidase depressed in neutrophils but its transfer to the lymphocytic histiocytic system of cells was impaired and assumption of the positive peroxidase reaction in lymphocytes and hypertrophying lymphocytes in the important 8 to 12 hour stages was either sparse or lacking The finding that this cellular respiratory system so obviously handicapped was matched by absence of clinical



Fig 6 Case of Boeck s sarcoid Peculiar massed histiocytic response after ACTH 5¾ hour inflammation In the normal control neutrophils predominate at this stage x 2000

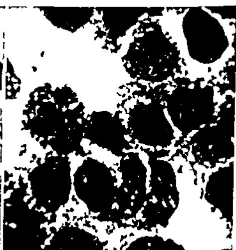


Fig 7 Case of degenerative bilateral chorioretinitis 10 hrs of inflammation Pre treatment exudate stained for verdoperoxidase Almost all cells including lymphocytes are strongly positive x 2000

response warrants further study Interestingly enough in the same two windows under discussion lymphocytes and hypertrophying lymphocytes participated in near normal numbers (Fig 8) In four additional windows in another patient lacking clinical response with respect to chorioretinitis although the verdoperoxidase mechanisms remained apparently intact lymphocytes and hypertrophying lymphocytes again remained in the post treatment windows

By contrast in four windows studied in a case of bilateral iritis with complete ACTH induced remission the verdoperoxidase mechanisms were qualitatively normal but there was in addition the extinction of lymphocytes and hypertrophying lymphocytes as usually observed during ACTH therapy

In those windows (Fig 9) in which the pretreatment response was characterized by a marked eosinophilic migration (periarteritis nodosa) the windows running concurrently with treatment showed diminution of eosinophils in areas of local experimental inflammation



Fig 8 Same case as Fig 7 10 hrs of inflammation Exudate stained for verdoperoxidase after ACTH Note diminution in enzymatic content Observe also failure of lymphocytic depletion x 2000

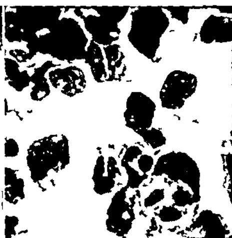


Fig 9 Case of periarteritis nodosa Pre treatment 7 hrs of inflammation Exudate rich in eosinophils Note lymphocytes Following ACTH eosinophilic component largely disappeared (see text) x 2000

GENERAL DISCUSSION

The changes observed in the local area of inflammation may well represent the summation of effects produced by the hormone not only at the local level but in addition at the levels of the peripheral blood the hematopoietic organs and the reticulo endothelial system of macrophages The conditions in the local area of inflammation in other words are subject to modifications dependent upon the conditions existing at the sources of supply of the cellular and humoral elements which ultimately migrate into the local area of inflammation

Although discussion of previously described changes in the defensive mechanisms at the levels of (a) peripheral blood (b) hematopoietic organs and (c) reticulo endothelial system of macrophages is beyond the scope of this present report it must be emphasized that changes in the local area of inflammation following hormonal treat

ment may reflect but need not necessarily correspond in all respects to changes at other listed levels of the defensive mechanisms

Finally it should be observed that in the evaluation of the cytologic response to hormonal therapy in patients two distinct sets of controls became necessary i e healthy volunteers and the patients before treatment Otherwise changes during treatment which can be rightly ascribed to defensive mechanism handicapped by pre existing disease might have been ascribed to the treatment itself while changes actually due to the treatment became apparent as such

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DISCUSSION

DR JOHN M MCLEAN (New York Hospital and Cornell University Medical College New York City) I would like to add one observation that is somewhat similar to these but by a slightly different method

If one injects a suspension of uveal pigment intradermally in the normal individual or in the individual with nonspecific ocular inflammation one gets a simple foreign body reaction upon biopsy done about two weeks later

If one injects the same uveal pigment suspension intradermally

in a patient with active sympathetic ophthalmia one gets a violent epithelioid cell reaction in the skin with complete or nearly complete phagocytosis of pigment

Recently we have observed that if we inject such a suspension *of uveal pigment intradermally in the patient with sympathetic* ophthalmia under ACTH therapy there is practically a complete suspension of cellular reaction and a complete absence of uveal pigment phagocytosis

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If one injects the same uveal pigment suspension intradermally

Before inoculation the animals were examined and their condition found to be healthy X rays and electrocardiograms were taken for comparative purposes

One cubic milliliter of a fecal suspension containing metacyclic forms of schizotrypanum was injected into the peritoneum of both puppies on the same day

Circulating blood was tested daily to prove the presence of try

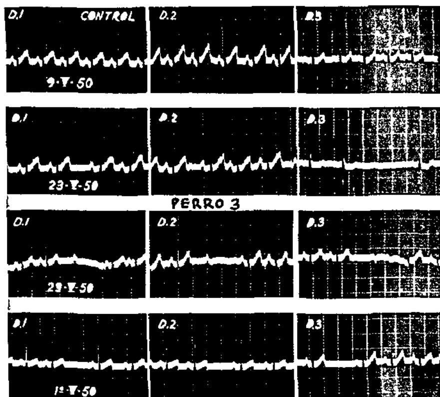


FIG 1 Electrocardiographic tracings of dog No 3 The first one was taken before inoculation the following after 14 20 and 31 days

panosoma It was positive on the seventh day in dog No 2 and on the ninth day in dog No 3

Electrocardiographic and radiologic studies were done daily which showed evident changes on the thirteenth day in dog No 3 (Fig 1) and on the tenth day in dog No 2 (Fig 2) These changes were second grade A V block in dog No 2 and a probably first degree A V block and a sinus arrest in dog No 3 (Fig 2) Heart enlargement was seen in dog No 3 (Figs 3 and 4)

Dog No 2 was chosen for ACTH therapy since he first showed evidence of the disease He was injected with ACTH on the four

45

Action of Adrenocorticotrophic Hormone in Chagas' Disease An Experimental Study

Javier Robles Gil, M Perrin and J Balcazar
RHEUMATOLOGY DEPARTMENT OF THE NATIONAL INSTITUTE OF CARDIOLOGY MEXICO

There are numerous investigations proving the favorable action of ACTH on inflammatory processes of different types and demonstrating that exudative and even proliferative tissue lesions of patients with rheumatoid arthritis rheumatic fever and other diseases of collagenous tissue improve markedly with ACTH [1,2] Finland[3] has observed similar effects on inflammatory lesions of the lung resulting from both bacterial and viral agents

In consequence we decided to study Chagas disease in which two types of alteration are notable in the heart On the one hand the acute inflammatory phenomena and on the other the severe fibroblastic reaction following the former Furthermore there is a certain degree of pathologic similarity between Chagas disease and rheumatic fever These facts together with the fact that ACTH inhibits to a certain extent proliferation of mesenchymatous tissue led us to experiment with its action in dogs previously inoculated with schizotrypanum

This investigation may be of interest not only because it is a means of combating this disease which as yet lacks an adequate therapeutic approach but also because it may contribute to the better understanding of the mode of action of corticosteroids in a number of diseases of varied nature

Although final conclusions are far from being reached this preliminary note is published with the purpose of presenting the seemingly favorable results obtained so far and of stimulating other investigators to study the problem directly in patients with Chagas disease which is uncommon in Mexico

MATERIAL AND METHOD

Two one and a half months old litter mate puppies were chosen weighing from 3 to 3 5 kilos They were labeled Dogs No 2 and 3

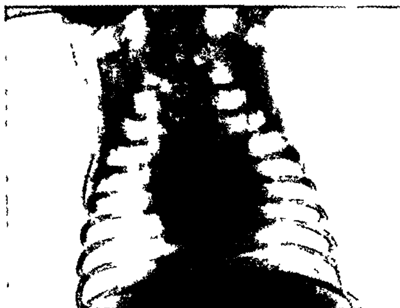


FIG 3 Chest X ray of dog No 3 before inoculation Cardio-thoracic ratio 0 50



FIG 4 Chest X ray of dog No 3 three days before he died Cardio-thoracic ratio 0 60

teenth day after inoculation with a subcutaneous dose of 10 mg divided into four doses of 2 5 mgs every six hours for three days After this 4 mgs a day were administered in divided doses for an additional two weeks

The progress of both dogs was carefully followed from the clinical

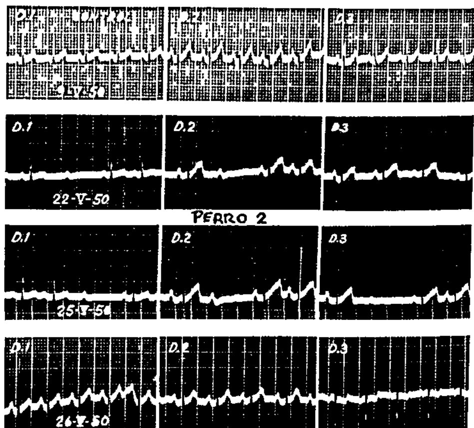


FIG 2 Electrocardiographic tracings from dog No 2 The first one was taken before inoculation the second and third after and the last one two days after ACTH treatment was begun

standpoint and also radiologically and electrocardiographically every three or four days

The course of the disease was quite different in the two dogs In dog No 3 the one not treated with ACTH the disease progressed rapidly and besides the symptoms mentioned there appeared diarrhea vomiting occasional convulsions generalized adenopathy edema ascites myocardial involvement and poor general condition progressing to a fatal outcome 33 days after inoculation

The course of myocardial lesions judged by X ray and electro


Fig 6 Chest X ray of dog No 2 before inoculation Cardio-thoracic ratio 0 48


Fig 7 Chest X ray of dog No 2 after inoculation and treatment with ACTH Cardio-thoracic ratio 0 49

cardiographic examinations was as follows On the fourteenth day after inoculation lengthening of the P R interval to 0 12 or 0 13 probable first degree A V block and also sinus arrest in dog No 3 (Fig 1) Three days later second degree A V blocks appeared in Dog No 2 and Dog No 3 together with blocked P waves all of which remained unchanged until the twentieth day Nine days later the second degree A V block disappeared but marked arrhythmia appeared (80–150) The next day the A V block reappeared persisting to the twenty ninth day after inoculation Two days before his death the last electrocardiogram (Fig 1) showed a total decrease in the voltage of QRS T and P and A V block of second degree due to

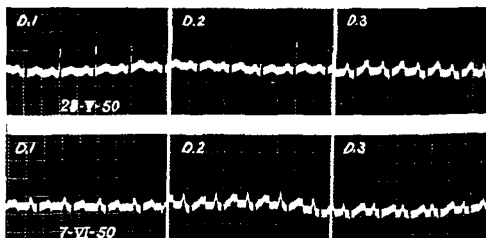


FIG 5 Electrocardiographic tracings from dog No 2 while treatment with ACTH was continued

probable Wenckebach Luciani periods with 0 11 to 0 13 P R intervals Figure 3 shows the heart before infection and Figure 4 taken two days before death shows heart enlargement especially to the right with signs of ascites

Dog No 2 treated with ACTH improved considerably Prostration anorexia and fever disappeared as well as lack of balance A certain degree of loss of weight and some ascites remained A V block observed on the tenth day after inoculation (Fig 2) disappeared on the second day of treatment the P R interval was reduced to 0 115 and eventually reached 0 095 whereas the rate increased to 158 or 180 Blocks did not reappear and the tracings were normal up to the time when the dog was killed for autopsy (Figs 2 and 5) The heart did not enlarge as is shown in Figs 6 and 7

When dog No 3 died dog No 2 was killed for comparative study of the lesions produced by Chagas disease in both cases In dog No

Fig 9 Heart lesions of dog No 3 with intramyocardial nests of Leishmania



Fig 10 Histopathological study of the heart from dog No 2 It is almost a normal myocardium with slight congestive lesions

a fatal bronchopulmonary infection and at autopsy the visceral lesions attributable to Chagas disease comparable to the control dogs were not present even though intramyocardial Leishmania nests were present

3 histopathologic studies showed severe acute myocarditis conges tion oedema and infiltration of monocytes and macrophages as well as plasmocytes lymphocytes and polymorphonuclear leucocytes (Fig 8) These infiltrations were diffuse interfascicular and of nodular granulomatoid disposition Pycnosis was usually accompanied by a homogeneous striated protoplasm and swelling and vacuolization of nuclei and protoplasm were also seen with wide perinuclear spaces in the hisian fascicular elements Intracellular nests are well shown in *Figure 9 of Leishmania*

Adrenal glands were congested showing profuse hemorrhagic lesions in the medullary zone with destruction

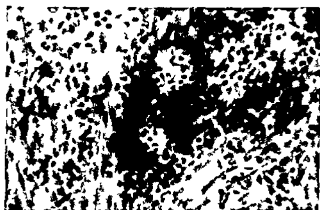


FIG 8 Myocardial lesions of dog No 3 due to Chagas disease Dense in flammatory infiltration with granulomatoid nodules

In dog No 2 some edema was present (Fig 10) in the myocardium with interfascicular capillary stasis and swelling of endothelial ele ments There was some diffuse mono lymphocytic infiltration but for granular infiltrations of hemosideri and some degeneration the myocardial cells were much less badly damaged than in the untreated dog Nonetheless small intracellular nests of Leishmania were pres ent Adrenal glands showed no hypertrophy or structurally important changes

From the histologic examination it was evident that the lesions were much less marked in dog No 2

In another experiment a dog treated with ACTH from the day of inoculation did not develop any of the signs or symptoms of Chagas disease and X ray and electrocardiograms did not disclose any cardiac lesions in spite of the repeated presence of trypanosoma in the blood Two untreated dogs inoculated at the same time devel oped the typical disease Unfortunately the treated dog developed

Fig 9 Heart lesions of dog No 3 with intramyocardial nests of Leishmania



Fig 10 Histopathological study of the heart from dog No 2 It is almost a normal myocardium with slight congestive lesions

a fatal bronchopulmonary infection and at autopsy the visceral lesions attributable to Chagas disease comparable to the control dogs were not present even though intramyocardial Leishmania nests were present

GENERAL DISCUSSION

Since this is a preliminary note brief non interpretative mention will be made of the outstanding features

Since the dog treated with ACTH showed such a favorable course this hormone apparently participated in some way in inhibiting the inflammatory process produced by schizotrypanum even though its presence in blood cultures and in injured tissues shows the absent corticoids do not act directly on the parasite The electrocardiographic radiologic and histopathologic changes are so evident that beneficial action of the hormone in Chagas disease in human beings seems beyond question Nevertheless confirmation should be obtained through further experiments in the treatment of human cases

It is yet to be ascertained if ACTH can prevent the fibroblastic *reaction of connective tissue of the Chagas disease heart or diminish* this reaction once it is present as in chronic myocarditis

It would also be useful to combine the action of ACTH with one of the known anti schizotrypanum drugs with the purpose of obtaining a radical cure

SUMMARY

The therapeutic effect of adrenocorticotrophin on experimental Chagas disease in dogs was studied A comparison has been made of the course of the disease with and without ACTH treatment

The results obtained are as follows

1 The clinical picture of Chagas disease in dogs treated with ACTH shows a striking improvement and the dogs remain alive

2 The clinical picture of the control animals with Chagas disease and *without treatment becomes very severe and the dogs die*

3 The myocarditis of the dogs under treatment improves considerably as compared to the control animals from a clinical electrocardiographic and radiologic standpoint

4 The histopathologic studies of the heart show more intense changes in the untreated dogs though in both cases there are foci of Leishmania in the heart

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4 Dorbecker C N Personal communications

DISCUSSION

DR MARCEL ROCHE I think Dr Robles Gil ought to be congratulated on his very nice work He has shown beautifully the effect that ACTH can have on the host parasite relationship in a tropical disease and thereby opens up a new field for this drug

I daresay that this is one of the rare times when ACTH has been used in an experimental disease before being used clinically It will be interesting to try this drug in clinical cases and particularly in Chagas Myocarditis which is fairly prevalent in Venezuela

DR JAVIER ROBLES GIL Thank you very much Dr Roche for your comments

Concerning the question of what happened to the parasites in the blood they continued to remain in the blood In the dogs treated with ACTH I found nests of Leishmanias in the myocardium so ACTH really has no effect on the parasite only on the inflammation and tissue changes which normally result from the presence of the organisms

Chagas disease is a very serious illness in experimental dogs We have had 80 or 90% deaths The other 10% of the dogs go into a chronic form of the disease with fibrosis of the myocardium and heart failure We have not done work in the chronic stage as yet but I think it would be less helpful than in the acute stage

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Further Observations on the Effects of ACTH in Acute Infections*†

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EFFECTS OF ACTH IN PNEUMONIA

In the first of these conferences data were presented which showed that ACTH administered to one patient with pneumococcal pneumonia and another with primary atypical pneumonia induced defervescence and remission of symptoms The latter patient with viral pneumonia suffered an exacerbation of symptoms when the hormone was discontinued Two additional patients with pneumococcal pneumonia and another with primary atypical pneumonia have since been treated with ACTH and observations on the two previously reported patients have been completed The findings have just been published in detail[1] and only the highlights of these cases will be summarized here

The first patient who was presented last year showed the following outstanding features 1) rapid defervescence following the institution of therapy with ACTH 2) prompt relief of symptoms with the drop in temperature 3) disappearance of evidence of toxemia at that time 4) bacteremia demonstrated first after ACTH was started that is at the time that crisis was induced and persisting while the patient was afebrile and free of symptoms 5) pneumococci persisting in the sputum and throat culture for at least 28 days after the patient had become afebrile 6) appearance of antibodies at the expected time and 7) failure of the lesion in the lung to extend and its rapid resolution during treatment The crisis in this case occurred while the patient was receiving 200 mg of ACTH daily and there was no relapse of pneumonic symptoms or signs when the dose was decreased or when the hormone was discontinued

* Aided by a grant from the United States Public Health Service
† The ACTH was supplied by The Armour Laboratories

The second patient a 41 year old man entered with type 2 lobar pneumonia involving the right middle and lower lobe The pneumonia progressed during the control period of observation For the first 12 hours after ACTH was started the patient s condition became progressively worse but this was followed by a sharp drop in temperature and the patient s symptoms then cleared rapidly The noteworthy features of this case are 1) the induction of a crisis following the administration of ACTH in a patient with bacteremia and consolidation of two lobes of the right lung 2) the persistence of rusty sputum for several days after the crisis 3) the persistence of pneumococci in the throat for at least one month after the crisis 4) absence of evidence of increased phagocytosis of pneumococci or bactericidal activity in the sputum after the clinical improvement 5) the appearance of antibodies relatively early but not unusually so for such an infection when not treated with ACTH 6) the resolution of the pneumonia which appeared to be neither accelerated nor delayed and finally 7) the failure of ACTH to affect the sedimentation rate significantly despite the rapid and favorable clinical response In this patient the major clinical improvement occurred while the patient was receiving 100 mg of ACTH daily there was no relapse of symptoms and resolution proceeded after the ACTH was stopped

The third case of lobar pneumonia was in a 48 year old man It was due to type I pneumococcus and involved the right upper lobe In this patient as in the others there was an initial early defervescence and relief from symptoms within 18 hours after the administration of ACTH was begun Unlike the previous cases however the fever in this patient recurred and symptoms reappeared while the patient was still receiving the same dose of the hormone namely 200 mg per day but a second drop in temperature occurred when the dose of ACTH was increased to 300 mg a day The fever returned again when the hormone was finally withdrawn The pneumococci in this case persisted in the sputum throughout the period of ACTH therapy and for at least one week thereafter The sedimentation rate remained elevated throughout the period of administration of ACTH This patient subsequently developed empyema Specific antibodies developed quite early in the course of this illness but at a time when they may be expected to appear in a case of type I pneumococcal pneumonia Eosinophiles which were uniformly absent in all three cases of pneumonia at the time the patients were first observed remained absent from this patient s blood for three days after the ACTH was discontinued In the other cases eosinophiles returned promptly when ACTH was decreased It is of interest that this patient developed angioneurotic edema due to hypersensitivity to

intravenously injected aureomycin several days after the ACTH therapy was discontinued

The findings in the first case of primary atypical pneumonia were still incomplete when presented at last year s conference In this patient a 26 year old housewife there was prompt defervescence and remission of all symptoms except for cough on a daily dose of 100 mg of ACTH There was then an escape from the ACTH as indicated by diuresis exacerbation of symptoms and return of eosinophiles in the blood but the fever again subsided when the dose of ACTH was increased to 200 mg a day This patient had viral pneumonia of unusual severity and there was slow resolution of the pulmonary lesion during the course of treatment with ACTH Cold agglutinins and streptococcus MG agglutinins appeared at the usual time and the ACTH did not affect the erythrocytic sedimentation rate Of additional interest in this patient was the fact that herpes labialis appeared while ACTH was being administered

The second case of primary atypical pneumonia was in a 15 year old girl and was characterized by prompt defervescence and remission of symptoms while the patient was receiving 100 mg of ACTH daily In this case there was slow but progressive resolution of the pneumonitis and there was no relapse of symptoms or extension of the pulmonary lesion when the administration of ACTH was stopped Cold agglutinins and streptococcus MG agglutinins appeared at the usual time This patient incidentally had severe acneiform lesions which did not appear to be accentuated by ACTH during the time that the hormone was administered

It would appear from these cases that ACTH altered the clinical course of pneumococcal and viral pneumonias The cellular mechanisms by which these alterations are produced require detailed experimental studies From the findings in these five cases we can conclude little in terms of the possible mechanisms by which the changes were produced The amount and time of appearance of antibody in each case was not unusual when compared with cases in which ACTH was not used

On the basis of the results observed ACTH is obviously not bactericidal It would therefore appear that ACTH so stimulated the host defensive activity or so altered the physiological responses that the usual manifestations of infection were obscured Some of the biochemical changes were studied in these patients and the findings were qualitatively similar to those which follow administration of ACTH in other conditions or in healthy individuals

The patient with empyema and the first case of viral pneumonia demonstrate that ACTH alone is not to be considered an effective therapy for pneumonia comparable to the antibiotics

EFFECT OF ACTH ON ACUTE INFECTIONS IN ANIMALS

Experiments have been carried out to determine the effect of ACTH on some infections in small laboratory animals In the case of pneumococcal infection of mice large doses of ACTH (2 5 mg every 12 hours) given before and after intraperitoneal infection with small numbers of virulent pneumococci failed to bring about survival of any of the mice Some of the animals receiving ACTH however tended to live a few hours longer than untreated control mice Similar findings have recently been reported by Glaser and his co workers with respect to streptococcal infections of mice and type I pneumococcal infections in rats[8]

Additional groups of mice were all given a dose of specific anti pneumococcal serum sufficient to protect them against 3800 MLD (minimum lethal dose) of type II pneumococci Subsequently these mice were challenged with varying doses of pneumococci and half of them were treated with ACTH before and after this challenge The mortality rates and survival times of the ACTH treated mice were the same as in the animals receiving the same infective dose and the same amount of antibody but not treated with ACTH

Similarly in the case of infection of mice with the PR8 strain of influenza virus the administration of ACTH failed to produce any significant alteration in the mortality rate

It was also of interest to determine whether ACTH would help animals to overcome the acute toxic effects of certain infectious agents Mice and rats were given doses of influenza virus which cause acute toxic deaths in these animals In neither case was there evidence that pre treatment with ACTH in any way increased the capacity of the mice or of the rats to withstand the toxic effect of large doses of the virus Animals receiving ACTH died after about the same interval following injection of the toxic agent as did control animals which received saline instead of ACTH

EFFECT OF ACTH ON FEVER

One of the implications of the clinical observations is that ACTH has some antipyretic effect This was clearly demonstrated to be the case Two patients were given increasing doses of typhoid vaccine either alone with aspirin or during ACTH administration In each of these patients it was shown on more than one occasion that ACTH diminished the intensity and duration of fever as measured by the area under the temperature response chart Similar findings were

also observed in rabbits challenged with either typhoidal or influenza viral vaccine as pyrogens

SUMMARY

In summary the effects which followed the administration of ACTH to patients with pneumococcal and viral pneumonia are dramatic but as yet unexplained

Obvious mechanisms of immunity namely antibody formation or release general resistance to infection and resistance to toxicity seemed not to be greatly affected by administration of ACTH[5]

Until more data are available dealing with the therapeutic usefulness of adrenal hormones in acute infections the possibility that they will mask important symptoms and signs of illness is so great and evidence of their value is as yet so slight that the hormones are not at this time to be recommended as useful therapeutic agents in infectious diseases

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DISCUSSION

DR JOSEPH J BUNIM In regard to the clinical effect of ACTH on pneumococcus infections I should like to report a rather disturbing experience we have had at Bellevue Hospital

This patient was a 52 year old white male who had typical advanced rheumatoid arthritis He was treated with cortisone for four

weeks and showed the usual satisfactory response During the fourth week of cortisone therapy he became much sicker and it was apparent that another disease had supervened

To clarify the nature of this second disease we discontinued cortisone We are still uncertain of the diagnosis but it is suspected that he probably had lupus erythematosus

Without any hormonal therapy the patient became progressively worse until he reached a critical period and it appeared he would die ACTH therapy (120 mg daily) was then instituted and again there was a very satisfactory response During the fourth week of ACTH therapy he suddenly developed overnight a temperature of 106° F became very toxic stuporous and died We did not know the cause of death

The autopsy findings consisted of 1) Changes in articular structures typical of rheumatoid arthritis 2) Marked hyperplasia of the adrenal cortex (the combined weight of the right and left adrenal glands was 29 grams) and confluent lobular pneumococcus pneumonia involving all five lobes Microscopically the alveoli contained fibrin numerous erythrocytes occasional mononuclear cells and numerous pneumococci Polymorphonuclear leucocytes were conspicuously scant The pneumonia had not advanced to hepatization and hence signs of consolidation were absent when the chest was examined one hour before the patient died

Dr Colin MacLeod identified pneumococci type IX from formalin fixed post mortem specimens of the lung and spleen

It is our impression that this patient died of an overwhelming pneumococcus infection during the fourth week of ACTH therapy and that the usual signs and symptoms of pneumonia were masked by the effects of the hormone

DR LAURANCE W KINSELL Dr Finland s observations last year impressed me as throwing a ray of light in a very obscure field in that for the first time one observed changes induced by ACTH in a disease of known etiology

His findings could be interpreted as indicating that the administration of ACTH or adrenal steroids either was capable of removing a variety of circulating toxins from the circulation or preventing the entry of toxins into cells or of doing something inside the cell to prevent the effect of the toxin upon specific enzyme systems

The following observation may throw some further light upon the site of action of these agents We have had the opportunity of observing one patient with tetanus who came in with the fully developed disease—a man who had already received huge doses of tetanus antitoxin He was having a generalized convulsive seizure at the time

we first saw him The first dose of ACTH was given about an hour later Within two hours there was definite evidence of general relaxation *In five to six hours he was able to sit up and to take food by* mouth

Over the next 24 hours he remained status quo but during the next 24 hours he lost ground While we were in the process of debating whether this represented a failure of adrenal response he had a fatal glottal spasm

At autopsy his adrenals were small i e they had apparently ceased to respond to ACTH for unknown reasons

It is generally accepted that tetanus toxin becomes fixed within body cells hence the failure to respond to tetanus antitoxin once the disease is developed If this concept is correct it would seem that in view of the preceding observations at least one part of the antitoxic mechanism of adrenal steroids must take place *within* the cell

DR FRANK L ENGEL We have confirmed Dr Finland s observation of *the inhibition of pyrogenic effect of typhoid vaccine by ACTH and* have made certain observations that present another tool for getting at this problem

We have found that typhoid vaccine when injected intravenously will produce a very *prompt fall in the plasma amino nitrogen* beginning in about two hours and becoming maximal in about six hours This fall occurs even if the fever is blocked by giving amino pyrine

In patients who are pre treated with ACTH and then given pyrogen and in whom fever is prevented the fall in amino nitrogen is much less ACTH itself does not alter the plasma amino nitrogen levels in acute experiments but prolonged ACTH therapy is associated with significant increases in plasma amino nitrogen Intravenous typhoid vaccine of course lowers the eosinophil count quite as effectively as ACTH We do not know the mechanisms of either the fall in plasma amino nitrogen after pyrogen or its inhibitions by ACTH

DR EDWARD FISCHEL We have not been as successful in inhibiting the pyrogenic reaction of humans to typhoid vaccine with ACTH or cortisone partly due to the well known variability of the febrile response However in rabbits whose reactions were well standardized by Ott s method and in another series of carefully documented animals Drs L Recant W Ott and myself could produce a moderate but statistically very significant inhibition of the febrile reaction to pseudomonas pyrogen and to pneumococcus vaccine

DR M JAMES WHITELAW (Good Samaritan Hospital Phoenix) In our observations on basal body temperature in women while under ACTH we have noticed that if one does not induce an amenorrhea the typical b b t is not altered We have done this on five cases

Another phenomenon we have observed which may have some bearing on the problem of the influence of ACTH on hyperpyrexia has been in our treatment of anemias Although there has been no apparent change in the haematological picture there has been a definite visual change in the peripheral vascular bed at the time diaphoresis was noted

These two isolated observations suggest that the action of ACTH may be peripheral instead of central in reducing hyperpyrexia This peripheral action might very well explain in part the results that one sees in the treatment of dermatological conditions as well as the phenomena noted in the tissue of a burned patient

DR MAXWELL FINLAND I have no comments but I would like to raise two questions that I am not able to answer decisively from what I have heard so far

First will ACTH or cortisone if given to a patient receiving antibiotics offer any additional help in overcoming the infection or in relieving the symptoms by masking or inhibiting the inflammatory response?

Second is it possible that in certain infections where there already is necrosis of tissue ACTH or cortisone may actually aggravate the underlying infection irrespective of whether or not antibiotics are used?

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Effect of ACTH on the Antistreptolysin O and Gamma Globulin Response to Hemolytic Streptococcal Infection (Scarlet Fever) and on the Development of Post-Streptococcal Sequelae*

Benedict F Massell, Edwin H Place George P Sturgis Morris Prizer, Joseph D Knobloch and Chang Shih Man

HOUSE OF THE GOOD SAMARITAN (CHILDREN S MEDICAL CENTER) AND SOUTH DEPARTMENT BOSTON CITY HOSPITAL BOSTON

Previous observations at the House of the Good Samaritan[1] revealed that the suppression of active rheumatic fever by ACTH is accompanied by a relatively rapid decrease in the concentration of gamma globulin and the titer of antistreptolysin O in the serums of patients in whom the gamma globulin concentration and the antistreptolysin O titer is at a high level at the time therapy is begun More recent observations suggest that rapid spontaneous recovery from severe rheumatic fever is accompanied by a relatively rapid decrease in antistreptolysin O titer whereas slow recovery is accompanied by a much more gradual decline in the level of this antibody The rate of decrease of the antistreptolysin O titer in the patients treated with ACTH is comparable to that observed in patients who recovered rapidly without specific therapy[2]

These observations do not indicate whether the decrease in streptococcal antibodies in ACTH treated patients is secondary (or coincident) to the suppression of the rheumatic process or whether the hormone therapy has a direct effect which in turn brings about improvement in the rheumatic process Therefore the present study was undertaken to elucidate this problem in mechanism The particular objective of the study was to determine whether ACTH therapy begun at the time of hemolytic streptococcal infection has any influence on the development of streptococcal antibodies and change in gamma globulin following such infection

* This study was supported by research grants from the National Heart Institute (United States Public Health Service) and the Helen Hay Whitney Foundation

CLINICAL MATERIAL AND PROCEDURE

The clinical material consisted of 20 patients with scarlet fever admitted consecutively last spring to the Contagious Disease Division of the Boston City Hospital Alternate cases were treated with ACTH The remaining cases were given only symptomatic treatment and served as controls Except for the administration of penicillin to 3 patients for short periods after their second week in the hospital *chemotherapy and/or antibiotic treatment was not used in this series* of 20 cases of scarlet fever

The ages of the ACTH treated patients varied from 5 years to 10 years and averaged 6 5 years The ages of the controls varied from 2 years to 10 years and averaged 6 6 years

In the 10 ACTH treated patients hormone therapy was begun very shortly after admission to the hospital and was continued in doses of 10 mg I M every 8 hours for a total of 14 days All of the patients (treated and controls) were observed in the hospital and/or in the clinic for 5 weeks or longer during which time serum was collected at frequent intervals throat cultures were taken for the determination of the presence of hemolytic streptococci and clinical and general laboratory examinations were made for the purpose of detecting any post streptococcal sequelae Group A hemolytic streptococci were isolated from the throats of all patients except in the case of one of the ACTH treated patients whose throat cultures remained consistently negative for hemolytic streptococci The group A strains were found to belong to type 3 in 6 of the ACTH treated cases and in 5 of the controls One of the throat infections in the treated group was due to type 14 and one in the control group was due to type 4 The remaining 6 strains (4 from control group 2 from ACTH treated group) could not be typed with the available serums Gamma globulin determinations were done on the sera drawn for antistreptolysin studies on ten of the ACTH cases and on seven of the controls The Looney modification[3] of the Kunkel[4] method was used

RESULTS

Antistreptolysin O Response

The initial and peak antistreptolysin O titers (presented in terms of logarithm of the titer) for each of the 10 ACTH treated patients and each of the 10 controls are shown by the scatter diagrams in Figure 1 The rise in titer above the initial titer for various intervals up to 5 weeks is indicated by the scatter diagrams in Figure 2 The average rise in titer for the ACTH group and the control group is graphically presented in Figure 3

Effect of ACTH on the Antistreptolysin O and Gamma Globulin Response to Hemolytic Streptococcal Infection (Scarlet Fever) and on the Development of Post Streptococcal Sequelae*

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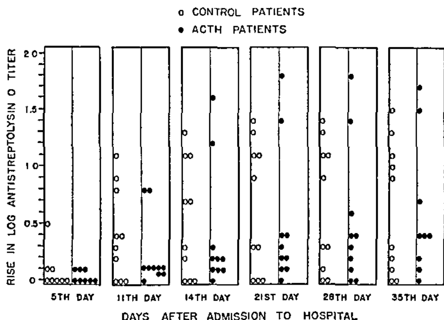


FIG 2 Distribution of rise in antistreptolysin O titers in 10 scarlet fever patients treated with ACTH and in 10 untreated controls

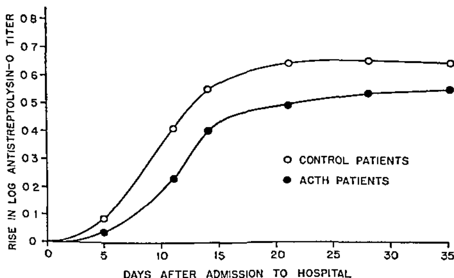


FIG 3 Average rise in antistreptolysin O titer in 10 scarlet fever patients treated with ACTH and in 10 untreated controls

Figure 3 shows that the average rise in titer for the controls was greater than for the ACTH treated patients but the difference is slight and statistically insignificant Furthermore from Figure 2 it is evident that there is considerable overlapping of the individual responses of the patients in the two groups A significant rise in anti streptolysin O titer (2 tubes or more) was observed within 5 weeks in

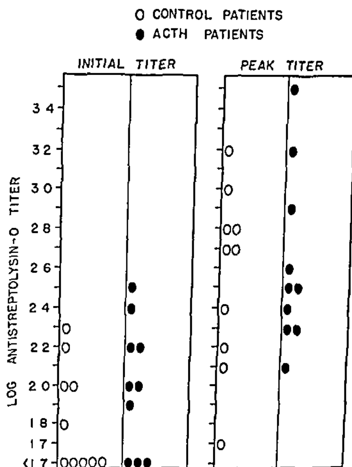


Fig 1 Distribution of initial and peak antistreptolysin O titers in 10 scarlet fever patients treated with ACTH and in 10 untreated controls

8 of the 10 ACTH treated patients and in 7 of the 10 control patients During the 2 weeks in which ACTH was being administered a significant rise in antistreptolysin O occurred in 6 of 10 patients receiving this therapy the rise amounting to 2 tubes in 3 cases 3 tubes in 1 case 12 tubes in 1 case and 16 tubes in 1 case The degree of rise during this period was comparable to that which had occurred at the end of the second week in the control group (see Figure 2)

Post Streptococcal Sequelae

Two of the ACTH treated patients and one of the control patients developed acute (post scarlatinal) glomerulonephritis The preceding throat infections in all 3 instances were due to group A strains of streptococci which could not be typed with available serums In addition one of the ACTH treated patients developed a fulminating attack of group A type 3 hemolytic streptococcal pharyngitis and pneumonia from which he died 48 hours after onset This fatal episode occurred 6 weeks after his initial attack of scarlet fever and 4 weeks after ACTH therapy had been completed The initial attack of scarlet fever seemed mild and was followed by a definite antistreptolysin O response Furthermore at a follow up visit 10 days prior to the fatal episode the patient appeared clinically to be quite well Re infection of course cannot be ruled out

No instances of rheumatic fever were encountered in this small series

CONCLUSIONS

ACTH therapy in doses of 30 mg daily for 2 weeks does not seem to influence the usual antistreptolysin O and gamma globulin response to streptococcal infection This finding in turn suggests that the mechanism by which ACTH suppresses the rheumatic fever process does not involve a reduction in streptococcal antibodies

ACTH in the doses used does not prevent the development of post streptococcal glomerulonephritis

The possibility that larger doses of ACTH or more prolonged hormone therapy may have effects other than those observed in this study cannot be excluded

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Gamma Globulin Response

The results show only relative changes in gamma globulin rather than absolute quantities since the turbidimetric readings have not been converted into grams All cases showed a rise in gamma globulin except two of the controls The three cases which developed nephritis showed the greatest and most prolonged rises A comparison of the average of the gamma globulin determinations in the two groups (excluding the three nephritis cases) showed no significant variation (Figure 4)

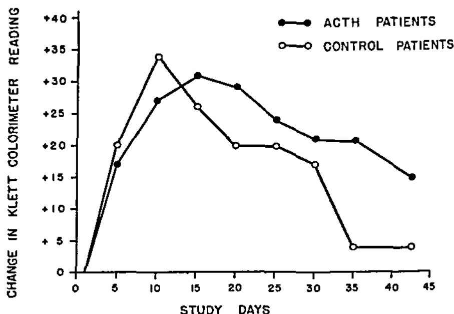


FIG 4 Average rise in gamma globulin in 14 scarlet fever patients 8 ACTH treated 6 untreated Three cases with complicating nephritis have been excluded

In this small series of cases a correlation of changes in gamma globulin and changes of antistreptolysin titer could not be demonstrated

Effect on Throat Flora

Bacteriological studies revealed that ACTH therapy had no apparent effect on the presence of hemolytic streptococci in the throat flora These organisms persisted in the upper respiratory tract of the ACTH treated patients to a degree comparable to that observed in the controls

Figures 5 and 6 illustrate the diminished antibody content of ACTH and cortisone treated animals when compared with simultaneously immunized control groups It is of interest that great variation exists in both the hormone treated and control groups but in toto a significant difference is present which is less however in the animals given only 2 5 mgm cortisone daily

When the cortisone is administered after immunization is well

The Effect of Cortisone on the Concentration of Ci culat g A tibody When the Hormone is Adm istered f m the Onset of Immun zation with Pneumococci

Mg antibody nitrogen pe ml. of serum

10 mg cortison daily			2 5 mg cortisone daily	
Rabb t	9th day	14 h day	Rabb t	14th day
	mg AbN/ml	mg AbN/ml		mg AbN/ml
B2	0 06	0 46	E24	0 49
B6	0 06	0 48	E8	0 62
B12	0 09		E29	0 67
B9	0 10	0 43	E20	0 70
B1	0 13		E28	0 80
B7	0 18	0 77	E26	0 85
B4	0 21		E23	0 85
			E5	0 85
			E27	1 06
			E7	1 19
Mea	0 12	0 55		0 81
Mean ±S E of groups without cortiso e	0 27 ± 02	1 47 ± 13		1 17‡ ± 06

0.27 and 1.47 are the average AbN/ml on the 9th and 14th days espectively of 27 rabb ts of Groups A a d C imm nized simulta eously with th B gro p animals I d vid al titers of these controls on th 9th d 14th days a e as f llow 0 13 0 49 0 28 0 54 0 19 0 60 0.21 0 61 0 07 0 69 0.31 0 75 0 22 0 99 0.32 1 12 0 23 1 15 0 19 1.20 0 16 1.26 0.36 1.33 0 28 1.34 0.27 1.54 0 40 1 54 0 22 1 57 0 37 1 61 0 12 1 68 0 35 1 68 0 22 1 95 0 17 2 16 0 16 2.23 0 27 2.34 0 52 2 40 0 43 2 52 0 31 3 05 0 47 (9th day only)

‡1 17 is the a erag a tibody nitrogen level per ml on the 14th day of 11 treated control an mals of G o p F mmunized s m ltaneously with th E group mals These controls had the following titers 0 41 0.52 0 71 0 85 1.04 1 11 1 19 1.34 1 46 2 04 2 18

Fig 6 (Courtesy Bjørneboe Fischel and Stoerk Jour Exp Med 93 37 1951)

established (Fig 7) a fall in concentration of antibody nitrogen is apparent in 6 of 10 animals within two or three days This experiment was repeated with similar results We are indebted to the Journal of Experimental Medicine for permission to reproduce the illustrations This work will appear in greater detail in the January 1951 issue of that Journal

Human antibody determinations usually are made with double dilution methods which may not detect changes of 50 to 100% An

4 Kunkel Henry G Estimation of alterations of serum gamma globulin by a turbidimetric technique *Proc Soc Exper Biol and Med*, 66 217–224 1947

DISCUSSION

DR EDWARD FISCHEL There are several factors to consider in the appraisal of the effect of any agent on the concentration of circulating antibody Of primary importance is the method of measuring anti

The Effect of ACTH on the Concentration of Circulating Antibody When the Hormone is Administered from the Onset of Immunization with Pneumococci

Mg antibody nitrogen per ml of serum at 14 and at 28 days after beginning immunization with pneumococci

After 14 days		After 28 days	
ACTH Group P	Control Group K	ACTH Group P	Control Group K
mg AbN/ml	mg AbN/ml	mg AbN/ml	mg AbN/ml
0 50	0 98	0 67	1 41
0 54	1 47	1 01	1 90
0 62	1 49	1 22	2 30
0 81	2 02	1 67	—
2 34	2 22	3 34	5 31
	2 30		4 72
	2 50		6 23
Mean 0 96	1 85	1 58	3 65

FIG 5 Five New Zealand red rabbits treated with ACTH approximately 0 5 to 1 0 mg Armour standard every 8 hours for the entire period 7 control rabbits of same strain (Courtesy Bjørneboe Fischel and Stoerk, Jour Exp Med 93 37 1951)

body and the wide variation in individual antibody production with a constant amount of antigen Even more variability exists with active infection which involves different amounts of many antigens Our experience with antistreptolysin O titers in patients treated with ACTH or cortisone is similar to that of Dr Massell and others

However an experimental approach to the effect of these hormones on antibody has been made with Drs M Bjørneboe and H C Stoerk using quantitative immunochemical methods Groups of rabbits immunized with polyvalent pneumococci were treated with hormone during the initial period of immunization and also after immunization was well established Antibody was measured by the quantitative agglutination technique of Heidelberger and Kabat (1934)

response in elaborating diphtheria antitoxin showed great quantitative variations but qualitatively was characterized by an increase in antibody detectable by the third to fifth day reaching a maximum between the tenth and twenty first days and declining thereafter ACTH was administered in different dosages and at variable times during the post inoculation period

Group I

Four patients who were treated for nine to fifteen days (80–100 mg ACTH/day in 4 divided doses) beginning within one hour after inoculation with toxoid (Table I) had qualitative patterns of response to the toxoid which did not differ from those expected of patients not receiving ACTH although two of them developed considerably less than the usually expected amount of antitoxin Another patient was treated with ACTH in amount of 20 mg every six hours for twenty days beginning on the seventh day after inoculation with diphtheria toxoid The amount of circulating antitoxin rose to a peak on the ninth day after inoculation with toxoid and then declined steadily during the next two weeks to a low level

Group II

Three patients received ACTH in amount of 20 mg every six hours for three or four days beginning fourteen days after inoculation with diphtheria toxoid (See Table I) Blood was obtained just before the first dose of ACTH and at frequent intervals thereafter for one or two weeks Two of these patients had a mild increase in circulating diphtheria antitoxin forty eight hours after ACTH was started but this diminished somewhat during the next two days and declined considerably during the ensuing ten days The third patient had a gradual steady decline in amount of circulating antibody beginning forty eight hours after the first dose of ACTH

Group III

Three patients received a single 40 mg injection of ACTH fourteen days after inoculation with diphtheria toxoid (Table II) Determinations of the amount of circulating antitoxin four hours after the administration of ACTH revealed no change in two patients as compared with the level of antitoxin found just before ACTH was given In the third patient there was a moderate reduction in amount of antitoxin after ACTH

Group IV

Eight patients were given 50 mg ACTH daily in a single dose for three days beginning twenty five or thirty days after inoculation with toxoid (Table II) Blood was obtained before and eight hours after

other possible cause of discrepancy with *human studies is that our* animals are hyperimmunized so that a relatively large proportion of circulating protein is antibody protein This may perhaps partake of the catabolic processes initiated by ACTH or cortisone more readily than do minute amounts of antibody globulin A striking decrease in the tagged protein molecules e g specific antibody globulin may occur within six hours (Fischel LeMay and Kabat J Immunol *61 89 1949) to three days (Fig 7)* and therefore may be attributable to the catabolic effect of adrenal hormones However we are currently studying the effect of cortisone on the disappearance rates of

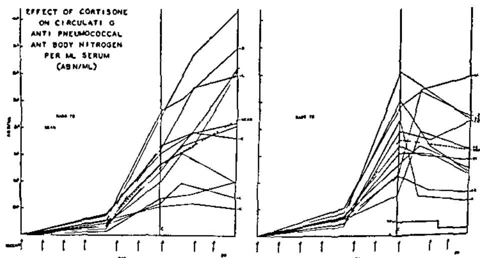


FIG 7 Effect of cortisone on circulating anti pneumococcal antibody nitrogen per ml serum (AB N/NL) (Courtesy Fischel LeMay and Kabat J Immunology 61 89 1949)

passively administered antipneumococcal antibody There appears to be no difference in the disappearance rates of antibody globulin administered to normal or to cortisone treated animals

In the actively immunized animals an inhibition of protein synthesis or antibody globulin formation is suggested by the striking morphologic changes that Dr Stoerk has observed in the *reticulo* endothelial and lymphoid tissues of these rabbits

DR W PAUL HAVENS JR (Jefferson Medical College of Philadelphia Philadelphia) During investigations of the capacity of patients with chronic hepatic disease to produce antibody an opportunity was presented to determine the effect of ACTH on the amounts of circulating diphtheria antitoxin *which appeared as an anamnestic response in Schick negative* patients at various intervals after the intramuscular inoculation of 50 Lf *purified* diphtheria toxoid The pattern of

Table II

Group	Case	*Units of Diphtheria Antitoxin/ml Serum on Various Days After the Administration of 50 Lf Diphtheria Toxoid*								
		14		*25*		*27*	*30*		*32*	*35*
		8 a m	*12 Noon*	*8 a m*	*4 p m*	*4 p m*	*8 a m*	*4 p m*	*4 p m*	*8 a m*
III 40 mg ACTH given at 8 05 a m on 14th day after injection of toxoid	1	363	363							
	2	121	85							
	3	170	170							
IV 50 mg ACTH given at 8 05 a m daily for 3 days beginning the 25th or 30th day after injection of toxoid	4			241	187	187	143			
	5			250		190	190			
	6			207	207	187	165			
	7						130	130	130	120
	8						26	26	26	24
	9						4 5	3 5		3 5
	10						385	363	363	363
	11						130	130	130	130

Table I

Group	*Case*	*Therapy with ACTH*		*Units of Diphtheria Antitoxin/ml Serum on Various Days after Administration of 50 Lf Diphtheria Toxoid*							
		Total dose mgm	*Duration days*	*10*	*14*	*16*	*17*	*18*	*21*	*23*	*28*
I 20–25 mgm ACTH q 6 hours was started 1 hour after toxoid was injected	1	680	9	60	130		170		170		
	2	680	9	50	50		50				
	3	1245	15	7	15				15		27
	4	700	9	150	250		210		150		121
II 20 mgm ACTH q 6 hours was started 14 days after toxoid was injected	5	240	3	190	240	270	230			140	
	6	320	4	24	28	24	22	22		14	10
	7	240	3	530	730	780		760			340

48

The Influence of ACTH on the Reactivity of the Bronchial Tree, Skin, and Secretory Glands to Specific Antigens, Histamine and Mecholyl in Bronchial Asthma*

David Grob, Edyth H Schoenrich Walter C Winkenwerder and A McGehee Harvey

JOHNS HOPKINS HOSPITAL AND JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE BALTIMORE

In an effort to obtain some information concerning the mechanism of action of ACTH in allergic disease the effect of ACTH administration on the reactivity of various tissues to a number of stimuli has been studied The tissues studied included one directly involved by an allergic disease the bronchial tree of patients with asthma and several that were not directly involved including the skin blood vessels of the skin and sweat salivary and gastric secretory glands (Table I) Three types of stimulating substances were administered (1) specific antigens extracts of pollens or spores (2) histamine and d tubocurarine which has the property of releasing a histamine like substance from the tissues and (3) the cholinergic agent mecholyl

Table I

Tissues Studied		*Effect of ACTH on Reactivity of Tissues to Following Stimuli Studied*
Those Involved by an Allergic Disease	*Those Not Involved by Allergic Disease*	Specific antigens (pollens spores, dust feathers)
		Histamine
Bronchial tree	Skin and blood vessels (immediate reactivity)	Histamine like substance released by contact with d tubocurarine
	Sweat salivary and gastric secretory glands	Mecholyl

* It is a pleasure to acknowledge the valuable technical assistance of Miss Grace Saltzer and Miss Barbara Ziegler in these studies We are also indebted to Dr John R Mote of Armour and Company for providing the ACTH that was employed

the first dose of ACTH and eight and seventy two hours after the last dose The amount of circulating antitoxin was slightly reduced in three patients eight hours after the first dose of ACTH and in four patients it was reduced eight hours after the third dose When tested three days after the last dose of ACTH seven out of eight patients had a mild to moderate reduction in amount of circulating antitoxin

It is evident that patients receiving ACTH for as long as fifteen days beginning within the first hour after the administration of a booster dose of diphtheria toxoid were able to make large amounts of antitoxin The amounts of circulating antibody found at various times after the administration of ACTH suggest that the administration of this material early (first to fifteenth day) in the period of development of antibody when the titer of antitoxin was *increasing* and later (twenty fifth to thirtieth day) at a time when the titer of circulating antitoxin was *declining* did not alter the pattern of response qualitatively as compared with the control subjects A quantitative difference in intensity of response could not be determined from these data

With one exception (Case 2 Table II) the reductions in amount of circulating antitoxin which occurred in four out of ten persons four or eight hours after a single dose of ACTH (40–50 mgm) were too slight to warrant the assumption that ACTH might have produced them although the possibility that a blocking or catabolic action was operative requires further investigation

The stimuli used in studying the reactivity of the bronchial tree were inhaled extracts of pollen or spores inhaled and intramuscular histamine and intramuscular mecholyl As Curry has shown[1] these substances are capable of producing in asthmatic patients an asthma like attack with reduction in vital capacity and maximal breathing capacity Graded concentrations of these substances were administered in order to ascertain the amount that would induce an asthma like attack These concentrations were then injected intramuscularly or inhaled deeply from a nebulizer under constant pressure of oxygen with eight deep breaths being taken in succession each held for 10 seconds The decrease in vital capacity and maximal breathing capacity which followed the administration of each agent was determined at frequent intervals The reactivity of the bronchial tree was expressed in terms of the greatest fall in these measurements of pulmonary function that occurred in each instance

It is readily apparent that many variables in addition to bronchial reactivity are capable of influencing the measured decrease in pulmonary function including depth of inhalation of nebulized solutions rate of absorption of intramuscularly administered compounds and the patient s respiratory effort during the measurement of pulmonary function Furthermore the reactivity of the bronchial tree to stimuli may be expressed in terms of the duration as well as the magnitude of decrease of pulmonary function These variables make difficult the assessment of minor changes in bronchial reactivity and necessitate caution in the interpretation of even major recorded changes especially with regard to measurement of vital capacity which is a less accurate index of bronchoconstriction and pulmonary function than is the maximum breathing capacity In three of the patients attacks of asthma could be induced at will by the inhalation of a specific antigen (extracts of ragweed or spores) while in the other two patients no reduction in pulmonary function could be demonstrated after the inhalation of any of a number of antigens including various pollens spores dusts and feathers In all five patients the inhalation of suitable concentrations of histamine and the intramuscular injection of histamine or mecholyl resulted in diminished pulmonary function and subjective and objective manifestations of asthma

The administration of ACTH was promptly followed by a definite diminution in the reactivity of the bronchial tree to inhalation of the specific antigens The duration of this diminished reactivity and the relation to the daily dose of ACTH varied in the three patients In one patient (G R Fig 2) there was a progressive diminution in the response of the bronchial tree to each of two concentrations of inhaled spores during the entire period of ACTH adminis

The reactivity of the bronchial tree to these stimuli was studied in five patients with seasonal bronchial asthma before during and after the administration of ACTH On admission to the hospital these patients all had moderately severe asthma In order to alleviate their symptoms and to improve and stabilize their pulmonary function they were kept in an air conditioned environment throughout the period of study In three patients the basal pulmonary function as measured by the vital capacity and maximal breathing capacity (maximal minute ventilation) was nearly the same during each of the three periods in which the reactivity of the bronchial tree was studied while in the other two patients it was appreciably greater during the last two periods of study (Fig 1)

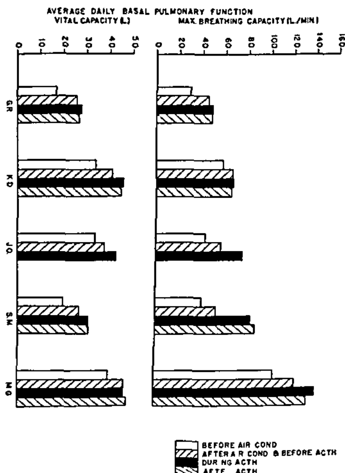


Fig 1 The influence of air conditioned environment and of ACTH administration on the vital capacity and maximal breathing capacity of five patients with seasonal bronchial asthma

This patient had a return of mild symptoms of asthma within seven days after ACTH was discontinued. The correlation between dose of ACTH and diminished reactivity to antigen observed in the last two patients suggests that the administration of larger doses of ACTH or of smaller doses of antigen might have produced more sustained suppression of reactivity.

The influence of ACTH administration on the reactivity of the bronchial tree to inhaled histamine was not as consistent as in the

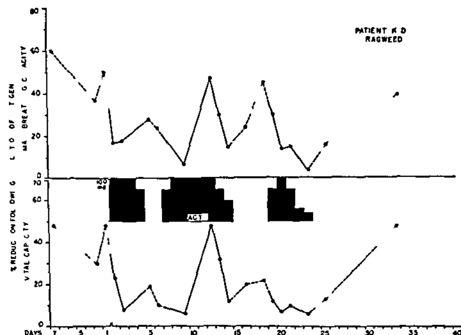


FIG. 3. The effect of ACTH administration on the bronchial reactivity of patient K. D. to inhaled extract of ragweed (0.04 mg. protein N/cc.)

case of inhaled specific antigen (Fig. 5). Two different concentrations of histamine were administered to each patient, varying from 0.05 to 0.50 mg./cc. In the first patient (G. R.) there was a slight decrease in bronchial reactivity during the period of ACTH administration. In the second patient (K. D.) there was a moderate decrease in reactivity during each period of ACTH administration, particularly to the higher concentration of inhaled histamine, and a tendency for the reactivity to return in the intervals between. In the third patient (J. O.) there was an increase in reactivity during ACTH administration. In the two patients in whom asthma was not induced by the inhalation of antigens but was induced by the inhalation of histamine

tration even though the daily dose of ACTH was gradually lowered from 100 to 20 mg Following cessation of ACTH therapy and discharge from the hospital the patient continued to have markedly decreased responsiveness to the inhaled antigen At the same time she remained entirely free of asthma at a time of the year when she had invariably experienced severe asthma in the past In the second patient (K D Fig 3) there was likewise a diminution in the response to inhaled antigen during ACTH administration However there

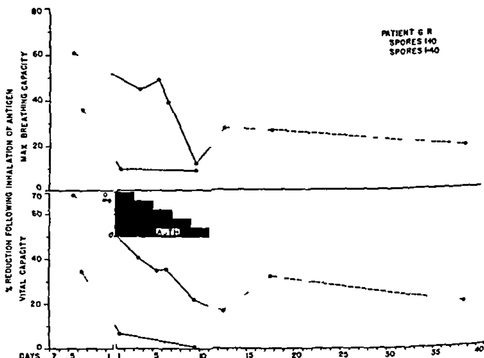


FIG 2 The effect of ACTH administration on the bronchial reactivity of patient G R to two concentrations of inhaled extracts of mixed spores (0 009 and 0 035 mg protein N/cc)

was a tendency for the responsiveness to return on one occasion while the patient was receiving ACTH and on three occasions after ACTH administration was interrupted or stopped This patient in contrast to the first had a return of the symptoms of asthma within three and five days after ACTH was discontinued on two separate occasions In the third patient (J O Fig 4) the response to inhaled antigen was reduced when 100 mg of ACTH was administered daily but returned rapidly whenever the dose of ACTH was lowered Restoration of the daily dose of ACTH to 100 mg was immediately followed by diminished reactivity of the bronchial tree to the antigen

two patients (J O and M G) with the decrease being transient in one of these (M C) while in the other two patients (K D and S M) the reactivity increased (Fig 8)

While it may not be entirely valid to summate the results obtained in different patients it is of interest that when this is done the average figures parallel to a considerable degree the overall impression obtained from consideration of the individual patients Thus

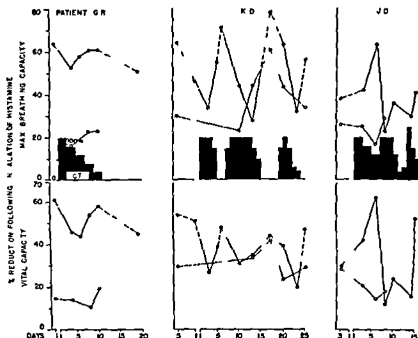


FIG 5 The effect of ACTH administration on bronchial reactivity to inhaled histamine Patients G R and J O inhaled 0 17 mg/cc (below) and 0 5 mg/cc (above) of histamine hydrochloride and patient K D 0 05 and 0 15 mg/cc The concentration of histamine is expressed in terms of the base

averaging the data obtained in all five patients (Fig 9) appears to show that the administration of ACTH was in general accompanied by (1) a moderate decrease in the reactivity of the bronchial tree to the specific antigens (2) a slight decrease in the reactivity to inhaled histamine (3) no appreciable effect on the reactivity to intramuscular histamine as reflected by measurement of maximal breathing capacity though a slight decrease as reflected by measurement of vital capacity and (4) a slight increase in the reactivity to intramuscular mecholyl Since the reduction of maximal breathing capacity is a more reliable measure of broncho constriction and decreased pul

there was a slight diminution in bronchial reactivity to the latter during ACTH administration (Fig 6) One of these patients (S M) showed decreased reactivity as measured by vital capacity determination but not as measured by maximal breathing capacity, which is a better index of pulmonary function The overall effect of ACTH on reactivity to inhaled histamine would appear to be a tendency to decreased reactivity, though not as marked as in the case of inhaled ex

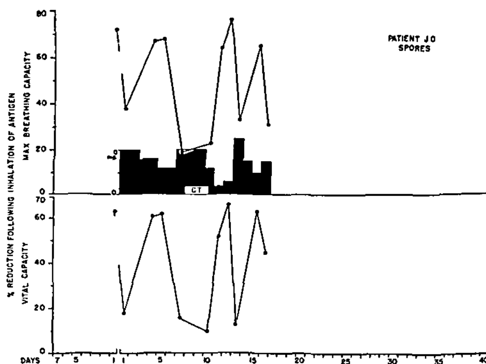


FIG 4 The effect of ACTH administration on the bronchial reactivity of patient J O to inhaled extract of mixed spores (0 1 mg protein N/cc)

tracts of pollen or spores and usually beginning after a longer period of administration of ACTH

The administration of ACTH did not appear to have any significant effect on the reactivity of the bronchial tree to intramuscular histamine (Fig 7) Two different doses of histamine were administered to each patient varying from 0 1 to 0 3 mg In one patient (J O) there was a slight decrease in bronchial reactivity during ACTH administration but in the other four patients there was either no appreciable change or a slight increase in reactivity particularly as reflected by the measurement of maximal breathing capacity

Following the administration of ACTH the reactivity of the bronchial tree to intramuscular mecholyl (5 to 9 mg) decreased in

ten patients with hay fever and in eight other patients with asthma rheumatoid arthritis lupus erythematosus nasal polyps or chorio retinitis The non allergic tissues that were studied included the skin blood vessels of the extremities and the sweat salivary and gastric secretory glands The reactivity of the skin to the specific antigens concerned with extrinsic asthma and hay fever (pollens spores dusts feathers) and to histamine and d tubocurarine was determined by measuring the size and in some instances the duration of the wheal

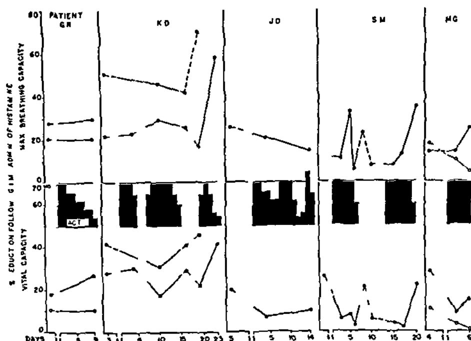


FIG 7 The effect of ACTH administration on bronchial reactivity to intramuscular histamine Patient G R received 0 25 and 0 30 mg of histamine hydrochloride patient K D 0 10 and 0 17 mg patients J O and S M 0 10 mg and patient M G 0 10 and 0 20 mg

and flare that immediately followed the intracutaneous injection of graded doses of these substances[8] The reactivity of the skin and vascular tree of the extremities to d tubocurarine was determined by injecting this compound intra arterially measuring the resulting rise in the skin temperature of the injected extremity and of the other extremities and observing the degree of local redness edema and wheal formation Systemic manifestations such as flush warmth and increased secretion of gastric acid were also studied The reactivity of the sweat and salivary glands and of the skin arterioles to intramuscu

monary function than is the reduction in vital capacity greater emphasis should probably be placed on the results reflected by the former measurement

The finding that the administration of ACTH for 10 to 17 days in doses sufficient to produce a remission of clinical asthma did not block the action of intramuscularly administered histamine or mecholyl on the bronchial tree supplements the report of Curry[2] that a single injection of ACTH which did not ameliorate the symptoms

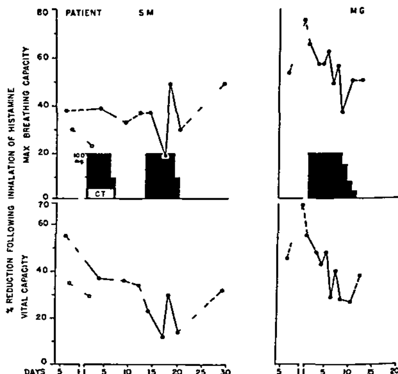


FIG 6 Continuation of Fig 5 Patient S M inhaled 0 25 and 0.50 mg /cc. of histamine and patient M G 0 15 mg /cc.

of asthma did not protect against the action of intramuscular histamine or mecholyl The studies that were made do not reveal the mechanism by which ACTH rapidly reduces the reactivity of the bronchial tree to specific antigens but they do illustrate one method of approaching this problem and show some interesting relationships between the dose of ACTH and bronchial reactivity to inhaled antigens

The influence of ACTH on the reactivity to these stimuli of tissues not directly involved by any allergic or hypersensitive disease was studied in the five asthmatic patients who have been discussed in

ten patients with hay fever and in eight other patients with asthma rheumatoid arthritis lupus erythematosus nasal polyps or chorioretinitis The non allergic tissues that were studied included the skin blood vessels of the extremities and the sweat salivary and gastric secretory glands The reactivity of the skin to the specific antigens concerned with extrinsic asthma and hay fever (pollens spores dusts feathers) and to histamine and d tubocurarine was determined by measuring the size and in some instances the duration of the wheal

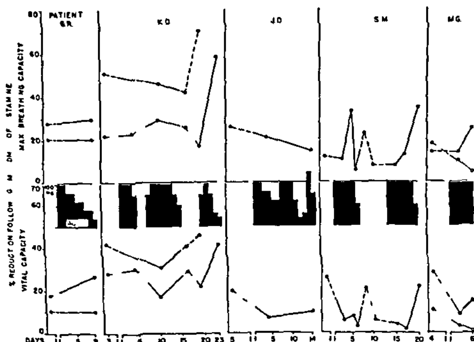


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larly injected histamine and mecholyl was estimated by observation of the degree of sweating salivation flush warmth transient fall in blood pressure and in some instances the headache or bradycardia produced by these compounds

The administration of ACTH in doses up to 100 mg a day for days or weeks had *no* appreciable effect on the reactivity of these non allergic tissues to the specific antigens studied or to histamine

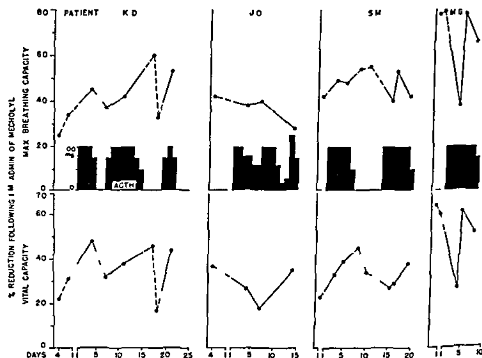


Fig 8 The effect of ACTH administration on bronchial reactivity to *intramuscular mecholyl (acetyl beta methylcholine chloride)* Patient K D received 6 mg mecholyl patient J O 5 mg patient S M 9 mg and patient M C 8 mg

d tubocurarine or mecholyl as determined by the methods described (Table II) The lack of influence of ACTH on the *immediate* intra dermal reaction to the specific antigens studied is in contrast to its known inhibitory effect on the *late* intradermal reaction to tuber culoprotein and other bacterial antigens The lack of influence of ACTH on the local and systemic effects of intra arterially injected d tubocurarine is of particular interest since there is evidence that this compound does not produce its vascular effects directly but rather as the result of the liberation of histamine like substances from the tissues [5]

In summary (Table III) preliminary observations on the effect of ACTH on the reactivity of allergic (bronchial) and non allergic tissues to a number of stimuli have shown that the administration of ACTH promptly decreases the reactivity of the allergic tissue that was studied to specific antigens ACTH does not appear to reduce the reactivity of this tissue to mecholyl or to intramuscular histamine but does have a somewhat delayed effect in reducing reactivity to inhaled histamine

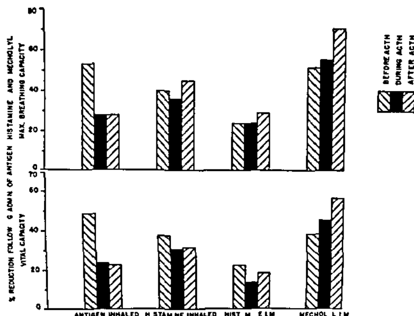


FIG 9 Summary and average of the data obtained in 5 patients (Figs 2–8) on the effect of ACTH administration on bronchial reactivity to inhaled antigen (extracts of pollens or spores) inhaled and intramuscular histamine and intramuscular mecholyl

On the other ACTH did not alter the reactivity of non allergic tissues to any of the stimuli studied

While these observations leave unanswered the very important question of how ACTH diminishes the responsiveness of allergic tissues to specific antigens they do indicate that in patients with bronchial asthma this action of ACTH is not mediated through alteration in reactivity to a cholinergic substance and probably not directly mediated through alteration in reactivity to histamine or in the release of histamine from the tissues although the latter possibilities are still in need of further exploration

larly injected histamine and mecholyl was estimated by observation of the degree of sweating salivation flush warmth transient fall in blood pressure and in some instances the headache or bradycardia produced by these compounds

The administration of ACTH in doses up to 100 mg a day for days or weeks had *no* appreciable effect on the reactivity of these non allergic tissues to the specific antigens studied or to histamine

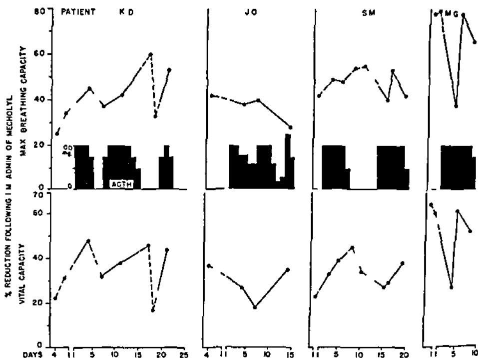


FIG 8 The effect of ACTH administration on bronchial reactivity to intramuscular mecholyl (acetyl beta methylcholine chloride) Patient K D received 6 mg mecholyl patient J O 5 mg patient S M 9 mg and patient M G 8 mg

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Table III

SUMMARY OF OBSERVATIONS ON THE EFFECT OF ACTH ON THE REACTIVITY OF TISSUES TO THE STIMULI THAT WERE STUDIED

			Effect of ACTH on Reactivity to			
			Specific Antigens (Pollens Spores Dusts Feathers)	*Histamine*	*Histamine like Substance Released by Contact with d Tubocurarine*	*Mecholyl*
Tissues Studied	Those Involved by an Allergic Disease	Bronchial Tree	Decreased	0?	0	0
	Those Not Involved by an Allergic Disease	Skin and Blood Vessels (Immediate Reactivity)	0	0	0	0
		Sweat Salivary and Gastric Secretory Glands		0	0	0

Table II

EFFECT OF ACTH ADMINISTRATION ON THE IMMEDIATE REACTIVITY OF SKIN TO SPECIFIC ANTIGENS HISTAMINE AND D TUBOCURARINE

Stimulus (0 05 cc intradermally)	Av Conc (mg /cc)	Pts Studied	Effect of ACTH on Reactivity of Skin: Unchan ed	Decreased			Increased		
			No of Pts	No of Pts	Avg Decrease in Reaction Wheal	Flare (min)	No of Pts	Avg Increase in Reaction Wheal	Flare (min)
Constant conc to each pt									
Specific Antigens	3×10^{-4} (PN)	12	6	4	0 7	2	2	0 6	3
Histamine	3×10^{-3}	18	13	2	0 4	1 7	3	0 2	2
d Tubocurarine	20	11	9	1	0 3	1	1	0 2	1
Graded Conc to each pt Threshold					Change in Threshold			Change in Threshold	
Specific Antigens	3×10^{-4} (PN)	12	4	4	10 ×		4	10 ×	
Histamine	3×10^{-3}	10	4	1	10 ×		5	10 ×	

maximum breathing capacities without inducing some degree of bronchoconstriction and discomfort in the sick asthmatic subject This test cannot be performed repetitiously (6 to 20 determinations in several hours necessary) in contrast to vital capacities in doing protection studies wherein one is trying to determine the improve ment which the therapeutic agent affords against the broncho con strictor effects of histamine methacholine or allergens

We have observed ventilometric and respiratory function before and after treatment in a number of asthmatic subjects It would ap pear that the greatest improvement noted is in the maximum breath ing capacity rather than in changes in the index of intrapulmonary mixing residual air or residual air to total lung volume ratio We have noted similar changes following successful physiologically di rected therapy Here again the improvement in pulmonary function and the improvement in histamine and methacholine sensitivity ap pear to be non specific

DR BRAM ROSE I would like to compliment Dr Schoenrich on her presentation of these findings which are of considerable interest In view of the fact that the response of the lungs to such agents as his tamine mecholyl and various antigens appears to be produced by different mechanisms the response of the young extrinsic asthmatic to such agents may differ from that of an older so called intrinsic asthmatic Perhaps experiments of this type may provide part of the answer as to the different characteristics of these two groups of asth matic patients

DR SAMUEL FEINBERG (Northwestern University Medical School Chi cago) The work that we did which touches upon the essayist s report concerns quantitative studies with histamine and antigens in allergic patients by quantitive intradermal nasal and ophthalmic tests The flare reactions on the skin from different concentrations of histamine in quantitating the reaction to histamine do not alter on repeated tests during ACTH therapy In our series of cases none showed any effect of ACTH on the histamine skin reaction

The antigens used to titrate the effect on the immunological re action were ragweed alternaria fungus house dust and horse dander In only one patient in the series was there any suggestion that ACTH may have diminished the skin reaction to antigens

In those asthma patients who had associated nasal and conjunc tival symptoms nasal and conjunctival tests were made with varying concentrations of ragweed mold and histamine With the exception of one patient there was no indication that there was any influence of ACTH on the responsiveness of these tissues The patient who did

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DISCUSSION

DR MAURICE S SEGAL (Boston City Hospital and Tufts College Medical School, Boston) The dyspnea and bronchoconstriction observed in sensitive subjects with histamine and methacholine are more striking and consistent when these agents are administered intravenously We did not employ the aerosol route with these agents in our ACTH studies The improvement in histamine and methacholine sensitivity observed in the course of treatment with ACTH is probably non specific inasmuch as we have observed similar improvement with a variety of other protective agents One striking change observed in one of our patients seems worthy of comment This girl was very sensitive to the effects of intravenous histamine and methacholine and aerosols of dog dander During the course of therapy with ACTH she demonstrated no protection against the effects of intra venous histamine and methacholine but extraordinary protection against the effects of dog dander aerosols

We have not observed any consistent changes in skin tests of the direct type and we have not been able to block successful passive transfer

The explanation for this variability in blocking by ACTH of the effects of a variety of stressors (histamine methacholine and allergens) at different target sites —skin and respiratory tract —is not apparent

The vital capacity test was routinely performed during the course of therapy in our patients because of its simplicity and our belief that it offers an adequate comparative guide to the patient s clinical course We are aware of its limitations in studying pulmonary pathophysiology Most of our patients were too ill for complete Cournand and Baldwin studies Furthermore it is difficult to perform frequent

pollen Each patient was given 20 mgs of ACTH intramuscularly every six hours for eight doses and the tests were then repeated It was of incidental interest to observe that the active discomfort of hay fever present in four of these subjects at the time of observation disappeared within a matter of hours after ACTH but recurred at varying intervals following the study

Figures 10 and 11 show the skin tests and the passive transfer tests performed before and after the ACTH therapy in these ten patients As may be seen there is little if any change in the immediate type skin sensitivity or circulating antibody titres observed in these patients on the dosages used in this 48 hour duration experiment

We have more recently observed a very severe asthmatic who re

PASSIVE TRANSFER TEST

	BEFORE ACTH	AFTER ACTH	
1	44 11	15	X
2	20	42 12	O
3	40 12	30	
4	20	20	
5	36 10	40 7	O
6	38 10	35	
7	50 10	40 10	
8	34 10	32 10	
9	30	12	X
10	20	30	O

X–DIMINISHED
O–INCREASED

FIG 11

show an effect was the same one whose skin test was modified Reagin titers studied in several patients were not affected by ACTH therapy

Therefore within the limitations of the investigation we fail to find any effect of ACTH on the skin or shock organ in spite of the obvious clinical improvement in the patient This seemingly paradoxical situation needs further investigation and experiments are under way to help resolve this disparity

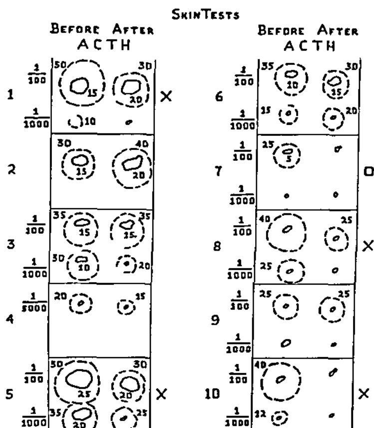


FIG 10

DR WALTER S BURRAGE (Massachusetts General Hospital and Harvard Medical School Boston) I wish to speak briefly in confirmation of the observations of Dr Schoenrich and of the previous discusser Dr Feinberg indicating that the immediate type of skin test is not affected by ACTH

We selected ten cases of typical seasonal hay fever showing large positive skin tests and passive transfer tests to the clinically offending

in control and treated groups when the latent period after passive sensitization was one half hour or 48 hours

Tuberculin Reaction

Twelve humans with carefully recorded P P D tuberculin reactions were given cortisone or ACTH for from two weeks to three months In instances of rheumatoid arthritis or rheumatic fever the dosage was sufficient to control the manifestations of these diseases and produce mild signs by hyperadrenalism Other patients were treated with larger doses for experimental purposes (viz 100 mgm ACTH daily for 1 month in three instances) Tuberculin reactions with P P D were repeated There was no change in the size or severity of the reaction during hormone treatment except in two of twelve instances In these two marked diminution or obliteration of the reaction occurred However these individuals manifested marked hyperadrenalism with striking changes in skin texture It seems more likely that non specific factors affecting the tissues were induced by the hormones than an actual inhibition of the allergic reaction per se

In guinea pigs immunized with dead tubercle bacilli the reaction to 0 005 mgm P P D tuberculin was the same in non hormone treated animals as in animals given 5 mgm of cortisone daily for 5 weeks

Streptococcus Nucleoprotein

The C18K fraction of Heidelberger and Kendall (1936) was injected before and during ACTH or cortisone treatment in eight patients No change was discernible in the appearance of the lesions during the period of hormone administration

These findings are in accord with those reported by Dr Rose and his associates at the first Armour Conference last year when it was observed that patients with asthma or hay fever may respond admirably to ACTH However the skin reaction to the offending antigen is not inhibited by such doses of hormone as are clinically effective

DR EDYTH H SCHOENRICH We all seem to be in agreement concerning the influence of ACTH on the reactivity of non allergic tissues such as the skin The differences which exist in the various findings reported today concern the studies of the reactivity of the bronchial tree in patients with asthma

Again I want to advise caution in the evaluation and interpretation of measurements of the reactivity of the bronchial tree It is extremely difficult to perform accurately these measurements of pul

ceived 1200 mgs of ACTH in nine days At the end of this course of therapy his scratch tests were strongly positive as they were before receiving ACTH

DR EDWARD FISCHEL ACTH and cortisone in large dosage may inhibit certain reactions of tissues to the adverse stimulus of antigen antibody union It does not seem likely however that antigen antibody union itself is affected It has previously been reported that several allergic reactions studied in humans and in animals are not affected by ACTH or cortisone Since that time our preliminary observations have been confirmed by further experiments In summary

Arthus Reaction

Arthus reactions were induced with known amounts of a single protein antigen (crystalline egg albumin) and known amounts of anti egg albumin Twenty two tests in guinea pigs and forty in rabbits given ACTH or cortisone were the same as an equal number of tests in simultaneously studied control animals This is true when amounts of antibody are used which resulted in severe as well as mild reactions In the guinea pigs doses of ACTH varied from as little as 1 mgm per day for five days to 2 mgm every 6 hours for two days preceding the induction of the reaction Several rabbits received 6 mgm ACTH in one dose 6 hours before inducing the reaction others 5 mgm every 6 hours for two days and in one experiment 4 rabbits received 0 5 to 1 0 mgm ACTH every 6 hours for 14 days enough to cause a significant decrease in spleen weight and in circulating antipneumococcus antibody The passively induced Arthus reaction to egg albumin remained the same as in non hormone treated control animals The active Arthus was not investigated since it involves two possible variables antigen antibody union and antibody concentration and the latter has been found to be depressed by cortisone or ACTH

Anaphylaxis

In agreement with the work of Leger Leith and Rose in which non-quantitative methods were employed the induction of anaphylaxis in twenty eight guinea pigs by a threshold amount of antibody and antigen by the method of Kabat and Landow (1942) or Benacerraf and Kabat (1949) was not inhibited by ACTH (2 mgm every 6 hours for 2 days) or cortisone (2 5 mgm daily for 2 days preceding the shock dose of antigen) Anaphylaxis occurred with equal severity

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Observations on the Metabolic Changes Resulting from the Administration of ACTH to Patients with Asthma and Allied Conditions*

Bram Rose, J A P Pare K K Pump R L Stanford K R Mac kenzie and Eleanor H Venning

ROYAL VICTORIA HOSPITAL AND MCGILL UNIVERSITY MONTREAL

During the past year studies on the effect of ACTH on vari ous forms of hypersensitivity were carried out on a group of 82 pa tients Fifty one were treated while in hospital and of these 18 were observed on the metabolic ward where balance studies were carried out The studies included urinary creatinine nitrogen vitamin C uric acid histamine histidine 17 ketosteroid gluco-corticoid chemi cal corticoid sodium chloride and potassium excretion In addition to blood studies which included hemograms and bone marrow bi opsy where indicated biochemical determinations and respiratory function studies including vital capacity maximum breathing ca pacity and residual air were carried out

Electrolyte balance studies were inconsistent there being reten tion of sodium and chloride as a general rule with associated in creased potassium excretion in some but this did not occur in others Generally the patients were in moderate positive nitrogen balance during the control period Following ACTH a negative balance oc curred in a few (Figs 1 and 2) but again this was variable The vita min C excretion was characterized by an initial peak of increased output on the first day of ACTH followed by a moderate decrease or return to control levels during the remainder of treatment (Fig 1 and 2) as described by Beck et al[1]

All cases had a moderate to marked excretion of urine histamine during the control period (Fig 1 and 2) Following ACTH this was usually increased in some subjects quite markedly although this was not invariable There was a general decrease in the output as therapy

The ACTH was kindly supplied from The Armour Laboratories Chicago The later supplies of ACTH and cortisone were made available by a grant from the Canadian National Research Council These studies were aided by a grant from the Canadian National Research Council

monary function in patients with asthma Our own results are certainly not conclusive and in carrying out our studies we came to recognize how many and varied the sources of error can be For example you will recall that in our pre ACTH values we found a wide range of reactivity to identical doses of the stimulating agents in any one patient

rather regularly that the older asthmatic shows a low excretion of gluco-corticoids and 17 ketosteroids (Fig 3) The administration of ACTH to this group of asthmatics resulted in an increase of the steroid excretion to within normal limits and occasionally slightly higher (Fig 3) In distinction to this group the younger group of extrinsic asthmatics where skin tests are positive and where the symp

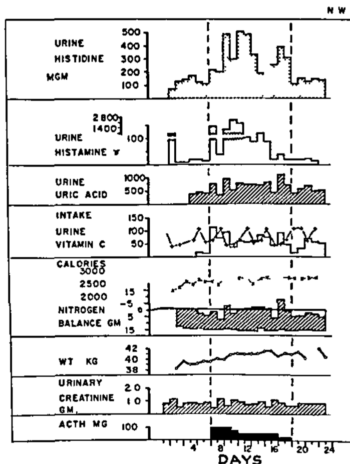


FIG 2

toms are due primarily to environmental factors showed a normal excretion of corticoids in the urine during the control period and a moderate to marked rise following the administration of ACTH It is difficult to say at this juncture whether these findings are valid because of the small number of patients studied but investigation along these lines is being continued

In considering the above findings with reference to other disease states which have been studied there appears to be only one

was continued and in the majority of cases the post treatment period was characterized by a reduction in the output of histamine as compared to pre treatment levels

Following the administration of ACTH the urinary histidine data demonstrated a consistent increase to levels comparable to those which are observed during pregnancy (Fig 2) On withdrawal of

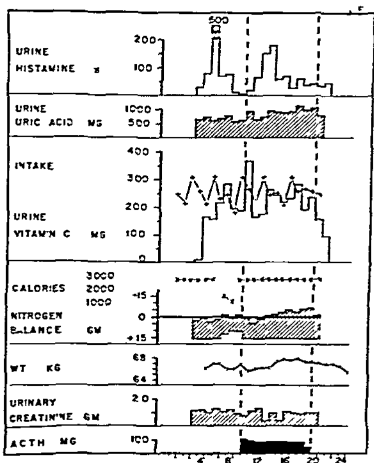


FIG. 1

ACTH the histidinuria decreased to within normal levels. Generally an increase in the uric acid output occurred following the administration of ACTH (Fig 1 and 2)

The pattern of steroid excretion has been discussed by Venning Rose and Johnson in another communication in this volume. A difference between the pattern of gluco-corticoid and the 17 ketosteroid excretion was apparent in the older intrinsic asthmatic, as compared to the younger so-called extrinsic asthmatics. It has been observed

It is of interest that one patient with vasomotor rhinitis mild asthma and diabetes mellitus was treated with ACTH on five different occasions during the past ten months A moderate increase in the impairment of the glucose tolerance over and above the initial diabetic level was observed but this was considered to be in keeping with the otherwise expected progress of diabetes in this patient

An example of these studies on a male patient aged 40 with extrinsic asthma will be found on the accompanying charts In Fig 4

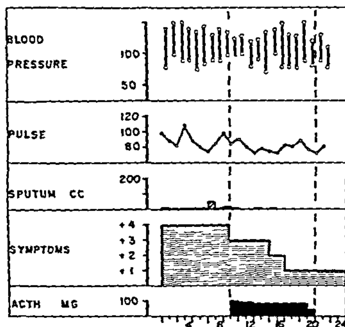


FIG 4

the blood pressure pulse sputum output symptoms and ACTH administration are shown It will be seen that there was little change in the blood pressure a moderate reduction in the pulse rate a virtual disappearance of the sputum output which was never great to begin with and a reduction in the symptomatology of the disease Fig 5 shows the respiratory rate which varied moderately and the tidal air which did not alter to any marked extent The residual air which was markedly increased during the control period being almost 80% of the total lung volume was reduced to 50% and finally approximated 42% by the end of the treatment This is compatible with a state of emphysema which was present in this patient. The maximum breathing capacity which was markedly impaired before

factor in the metabolic pattern observed which distinguishes the patients with asthma or other forms of hypersensitivity from other groups of patients and this concerns the excretion of histamine In a group of 5 control subjects in whom the histamine output of the urine was measured moderate amounts in the neighborhood of 50 micrograms per 24 hours were excreted In one of these subjects who

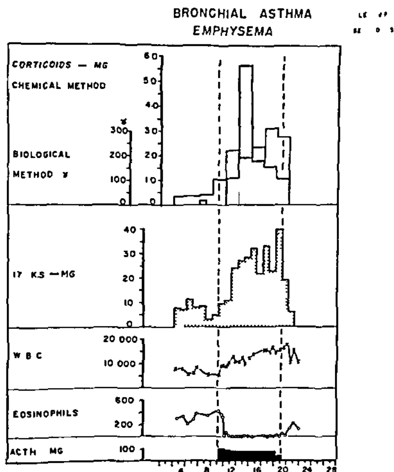


Fig 3

had developed a cold an increase to 1 000 micrograms per 24 hours was noted but this rapidly disappeared as the patient recovered from his cold *In contrast* to these results amounts equal to 250 000 micrograms (or 250 mgm) of histamine have been observed to be excreted by asthmatic patients during a 24 hour period The significance of these enormous amounts of histamine is not clear at this time It is evident that further studies will be necessary before any fixed pattern of metabolic change may be clearly defined

Figure 3 are shown urinary excretion studies of the chemical and biological corticoids as well as the 17 ketosteroids white count and eosinophiles In this patient who had a normal excretion of chemical and biological corticoids during the control period the marked increase in the corticoids following the administration of ACTH is obvious the chemical corticoids rising from 0 5 to 5 5 by the fourth day of ACTH administration and the biological corticoids rising from an average value of 50 to as high as 300 glycogenic units daily The 17 ketosteroids which were in the neighborhood of 10 during the control period rose to a high of 35 during treatment The usual increase in the white blood count and rapid decrease of the eosinophiles following ACTH is illustrated

In Figure 2 are the results of a similar metabolic study on a female patient aged 16 with asthma who was given ACTH for 12 days and in whom a prompt remission of symptoms occurred The upper portion of the chart shows the marked increase in the output of urinary histidine which rose from an average of 150 mgm per 24 hours before ACTH to 500 mgm per 24 hours during the administration of ACTH

These findings point to a deviation in the metabolism of histamine in these hypersensitive patients as compared to non allergic subjects and are in keeping with previous studies on the experimental animal in which an increase of histamine in the tissues was found following adrenalectomy [2]

There appears to be some justification for the concept that asthma of the intrinsic variety is in part due to a hypo function of the adrenal cortex On the other hand the evidence for this in the younger intrinsic group of asthmatics is not as apparent

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DISCUSSION

DR T G RANDOLPH (Northwestern University Medical School Chicago) It would seem that many of the beneficial clinical effects obtained from ACTH therapy occur in known allergic diseases in which the allergic factor is known to be present

treatment being only about 40% of the predicted value rose to about 60% by the end of the treatment as did the vital capacity Fig 1 shows some of the metabolic findings on this patient From above down will be seen the urine histamine uric acid vitamin C nitrogen balance weight and creatinine excretion It will be observed that there was a decrease in the histamine output in the urine which was in the neighborhood of 500 micrograms per 24 hours be

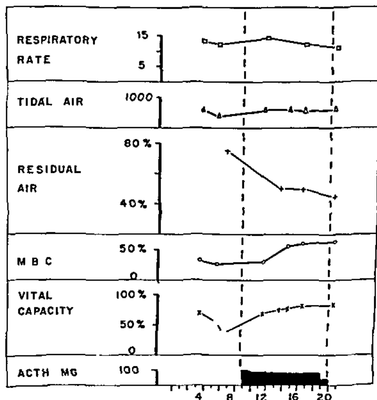


Fig 5

fore treatment and fell to approximately 50 micrograms per 24 hours by the end of the course of ACTH There was a slight increase in the urine uric acid The initial peak in vitamin C output will be seen immediately following the administration of ACTH followed by a reduction in the output of this substance The next line shows the nitrogen balance which demonstrates an increase in urine nitrogen following the administration of ACTH It will be noted that this patient gained in weight as did most patients receiving either ACTH or cortisone The changes in the output of urinary creatinine are not accounted for since the urine collections were rigidly controlled In

dicating that ACTH physiologically tends to reverse the character istic phenomena of allergy as postulated in the acid anoxia endocrine theory of allergy

Anoxia and edema may be the factors primarily responsible for the allergic physical fatigue syndrome and the allergic mental fatigue syndrome manifest to some degree in most allergic patients

Anoxia resulting from edema and sluding is believed by me to be the chief cause of pain and other diseases affected by ACTH such as myositis fibrositis and rheumatoid arthritis

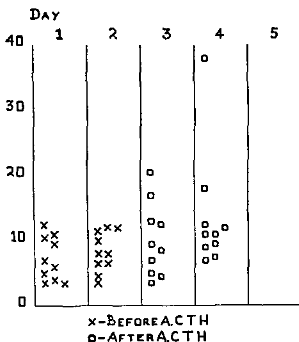


FIG 6

Dr L Ehel s presentation this forenoon indicating that ACTH therapy is associated with an increase of extracellular fluids is of par ticular interest in considering the acid anoxia endocrine hypothesis of allergy

DR WALTER S BURRAGE We have studied the 17 ketosteroid excretion in asthmatic patients before and during the administration of ACTH and as may be seen in Figure 6 the 17 ketosteroid excretion prior to ACTH was somewhat low in all of the patients

Of even more interest is the fact that the 17 ketosteroid excretion rose in only 6 of the cases and then to only a moderate degree al though the clinical response was good in all of the patients

Dr Harry G Clark and I have recently developed an acid anoxia endocrine working hypothesis as the basic mechanism of allergy I would like to explain this briefly and to theorize on the mode of action of ACTH in this connection As I see it (and I realize that much of the subject matter to follow may be controversial and difficult to substantiate or refute) since our knowledge of physiology at the cellular level is still meager Nonetheless I should like to present this hypothesis to this group to stimulate work at the fundamental level

The final stages of digestion occur intracellularly in man as in unicellular organisms This in our view may result in the production of predominately acid intracellular end products such as amino acids from proteins pyruvic and lactic acids from carbohydrates fatty acids and ketones from the breakdown of fats and carbon dioxide from all of these sources The presence of these intracellular acid products should attract *extra*cellular fluid into the cell as it is known that proteins and other colloids absorb water when they are acidified This should produce an intracellular edema

In the presence of this edema oxygen exchange of a cell may be diminished and the resulting anoxia may further increase the acidification of the cell Of interest in this connection is the fact that pH of the interstitial fluid and arterial plasma is 7 4 but venous plasma is 7 35 the blood having picked up acid end products at the cellular level The anoxia is further aggravated by the occurrence of sluding or the intravascular red blood cell agglutination phenomenon commonly found in acute allergic reactions These processes may produce a vicious circle providing they occur beyond a critical rate of speed

In the presence of a specific allergic reaction these intracellular mechanisms may occur at a greater rate of speed than can be buffered by the available electrolytes and perhaps by the alkaline granules of the eosinophils for it is known that the eosinophil cell is the first to be attracted to the site of the allergic reaction

This response should stimulate the pituitary adrenal system to greater activity but if the oxygenation of the endocrine organs is impaired by the existing allergic attack they may not respond maximally by elaborating mineralocorticoids Thus the patient s response may depend upon the degree of specific sensitization the dose of the allergen and the functional integrity of the endocrine glands with particular reference to the pituitary adrenal system

The tissues of a person in a chronic phase of allergy appear to be in a state of constant edema and anoxia which may be produced by the constant insult of an inhaled contacted or ingested allergen

The retention of sodium the mobilization of potassium the excretion of amino acids and the improvement of previously existing sluding are observed in many diseases affected by ACTH including chronic allergic manifestations I interpret these phenomena as in

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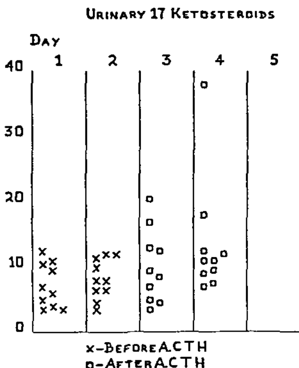


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also presented relating to kidney function in nephrosis and suggestive data are put forward concerning a possible physiologic defect in nephrosis In addition results are presented which suggest that electrolytes and electrolyte shifts have other physiologic effects quite apart from their role in the regulation of homeostasis of the body fluid compartments and the implication is raised that electrolytes may play a role in precipitating some psychoses Data are also presented concerning purely vascular effects of electrolytes as these are altered by adrenal cortical stimulation with particular reference to hypertension and congestive heart failure Furthermore data are presented showing changes in the tissue content of electrolytes under adrenal cortical stimulation thus further clarifying the interrelationship of sodium and potassium in the fluid compartments of the body Finally data are presented suggesting that corticosterone may be the major physiologically active corticoid in maintaining electrolyte and fluid homeostasis in the body In conclusion it is fair to say that considerable progress has been made toward a clearer understanding of the metabolism of electrolytes and the role of the adrenal gland and the kidney in maintaining homeostasis

Likewise further progress has been made in studying nitrogen or protein metabolism in more detail Thus data are presented to suggest that there is a difference between stress and ACTH in their effect on nitrogen metabolism and it has been shown that ACTH will correct the tyrosine metabolic defect in premature infants although there may be a concurrent decrease in the growth curve of such infants It is also reported that amino acid metabolism or turnover may be altered by adrenal cortical stimulation and that certain of the plasma proteins may be altered in some individuals by the same means In addition it is reported that the urinary excretion ratio of uric acid and creatinine may be substantially altered by dietary intake thereby clarifying some of the conflicting results observed in the adrenal cortical response test using ACTH

Of further interest are the results suggesting that adrenal cortical stimulation may alter the metabolic pathway of fats and fatty acids in both normal and diabetic patients to the end that sustained ketonemia may be circumvented The data also suggest that fats may be a substantial source of energy under adrenal cortical stimulation

Reports are also presented which suggest that adrenal cortical stimulation may alter vitamin A metabolism and ascorbic acid metabolism thus extending the role of the adrenal cortex to vitamin metabolism in general This is under further investigation

Another large area of importance at the fundamental level is the effect of adrenal cortical stimulation in liver disease and on the hemopoietic system in general Thus the course of acute hepatitis

Summary

A review of the results presented in this volume reveal that substantial progress has been made during the past year over a wide area of physiology and metabolism in both health and disease

Early in this volume results are presented suggesting that ACTH has a specific stimulating effect on the adrenal cortex only and that the so-called small molecule high potency ACTH has the same general physiologic adrenal cortex stimulating effects as has the so-called large molecule ACTH which is secreted by the pituitary gland

The data presented also reveal considerable progress in methods for studying adrenal cortical steroid secretory products and urinary steroid excretory products Thus it appears that the major biologically active corticoids secreted by the adrenal cortex are Compound F or hydroxycortisone and corticosterone Data also are presented suggesting that there may be different metabolic pathways of adrenal corticoids and that patients with different diseases may excrete certain steroid degradation products that are not excreted by most normal individuals The latter data suggest either the secretion of abnormal corticoids by the adrenal cortex or an abnormal metabolism of corticoids by the patient in question

Results are presented suggesting that in the experimental animal there is a hormonal interrelationship between ACTH and the growth hormone and that the latter may be an important component in certain experimental animal diseases

An area of substantial progress during the past year was the study of the effects of adrenal cortical stimulation on the intracellular and extracellular fluid compartments and their electrolyte contents as well as the migration of electrolytes between the two compartments and the excretion of electrolytes under different physiologic conditions

Results are presented which suggest that the electrolyte effect of adrenal cortical stimulation in pregnancy appears to be substantially the same as in the normal person whereas in the newborn electrolyte excretion does not appear to be materially altered by adrenal cortical stimulation Other data are reported which further clarify kidney glomerular and tubular function with particular reference to fluid and electrolytes and which suggest that there is what may be called an adrenal renal *interrelationship* Observations are

plement and extend beyond those reported in this volume This is particularly true of the physiologic phenomena associated with disease However the major emphasis in the second volume relates to the therapeutic utility of ACTH in dealing with different abnormal metabolic and physiologic states and in treating specific disease entities

Extending beyond the physiologic results presented in this volume and even in the second volume it may be added that many if not most of the symptoms and signs of illness in general may be inhibited or ablated if the adrenal gland is stimulated to this end by excessive doses of ACTH This must be kept in mind in treating patients since the occurrence of intercurrent disease may be completely masked if the patient is overdosed

In summary the past year has resulted in the widespread exploration of the role of the adrenal gland in physiology and metabolism and in health and disease It is fair to say that the scope of adrenal cortical function has extended far beyond that visualized only recently but even so the problem is far from defined

Phenomenal progress has been made but there is still a tremendous amount of work ahead first to define more clearly the role of the adrenal gland in health and disease in general then to rationalize what may be called a medical revolution and finally to resolve what may be the basic difference in mechanisms between health and illness in general and what may be the specific mechanisms operating in different specific disease states

When these ends are achieved a rational approach to this new era of medicine should be possible and specific means for treating these many apparently unrelated diseases should evolve

may be materially altered by ACTH and there are suggestions that adrenal cortical stimulation may allow the differentiation of some types of chronic liver disease Evidence is also presented which suggests that iron and porphyrin metabolism may be definitely altered by adrenal cortical stimulation and that pernicious anemia some of the experimental anemias of animals and some of the refractory anemias in man may be altered by adrenal cortical stimulation In the light of the results in this field to date it is fair to say that the adrenal corticoids probably play a vital role in liver function and in adequate function of the hemopoietic system as a whole Further studies are indicated and are in progress in this field

Studying these reports it is evident that the adrenal cortex plays a wide and important role in many aspects of metabolism and much more work is indicated to define more clearly the function of the adrenal cortex in nutrition and metabolism in general and in all of their aspects

In addition to the broad and profound role of the adrenal gland in metabolism ACTH and the adrenal corticoids have many more purely physiological effects some of which are presented in this volume and many more are reported in Volume II Therapeutics although all of the effects observed are not specifically emphasized

In this volume it is reported that under the influence of adrenal cortical stimulation or adrenal corticoids the acute inflammatory reaction in the eye may be inhibited not only to the instillation or injection of known antigens and bacteria but also to simple irritating chemicals such as glycerin

Other results are presented suggesting that the tissue response to other types of injury may be altered by the administration of ACTH or adrenal corticoids and that the clinical symptoms and signs of experimental Chagas disease in animals may be ablated by adrenal cortical stimulation although the causative organism remains in the tissues On the other hand other studies reported suggest that the course of experimental bacterial infections in animals is not altered by adrenal cortical stimulation Much more work will be required to clarify the role of the adrenal gland in infections in general in both animals and human beings

Data are also presented concerning the effect of ACTH on the development of antibodies The majority of the results reported re veal that under the conditions of the experiment and in the doses used ACTH had no substantial effect on the development of antibodies Finally results are presented relating to different hypersensitivity phenomena some of which are altered whereas others are not changed by adrenal cortical stimulation with ACTH

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When these ends are achieved, a rational approach to this new era of medicine should be possible, and specific means for treating these many apparently unrelated diseases should evolve.